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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Dietary Carbohydrate to Fat Ratio Influences Protein and Energy Utilization in Man and Animals* submitted by Linda McCargar in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Nutrition.

Date June 2, 1988

Dedication

in loving memory
of my Dad —

Lindsay Whyte

who, along with my Mom, Agnes Whyte,
and my family, has given me
continuous support and encouragement
all my life

Abstract

Experiments have shown that the non-protein source of dietary energy improves nitrogen retention. Whether this effect is greater with a high fat or a high carbohydrate diet has been controversial. The metabolic response to diets of different ratios of carbohydrate to fat was assessed in humans and animals. When adult male subjects were fed a diet high in fat with a carbohydrate to fat ratio of 1.0 (Cho:Fat 1.0), nitrogen balance (mg N/kg body weight) was improved compared to when a high carbohydrate diet (Cho:Fat 2.0) was fed. Similarly, when rats were fed a high fat diet (Cho:Fat 0.5), higher nitrogen balance values were observed (mg N/kcal energy gain), different from animals fed high carbohydrate diets (Cho:Fat 2.0, 2.5). These animals also had the highest carcass protein gain and the lowest carcass fat gain as a function of energy gain. The nitrogen-sparing effect demonstrated did not appear to be explained by hormone levels observed, or by changes in the rate of protein synthesis, but was due to adaptations in energy metabolism.

With high fat feeding, energy expenditure decreased in animals and humans, and fat oxidation increased in human subjects. With the availability of excess lipid fuels in the blood, alternate pathways of energy metabolism were utilized. When a greater proportion of fat was used for energy, more nitrogen was retained. When a greater proportion of carbohydrate was used for energy, more fat was retained. It is concluded that manipulation of the non-protein energy source in the diet influences both protein and energy utilization.

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Table of Contents

1. Literature Review **1**

Introduction 1
Metabolic Aspects of Protein Turnover 3
Digestion and absorption 5
Excretion 7
Synthesis 9
Degradation 12
Oxidation 12
Protein-sparing 13
Indicators of protein-sparing 13
Carbohydrate to fat ratio and protein sparing 14
Human and animal studies 14
Short-term effects of meal feeding 17
Enteral and parenteral feeding 18
A Mechanism for Protein-sparing 21
Hormonal effects 21
Insulin 21
Growth hormone 24
Androgens 24
Corticosteroids 24
Glucagon 25
Thyroxine 26
Substrate effects 27
Summary 29
References 30

2. Research Plan **37**

Rationale 37
Hypotheses 38
Objectives 38

3. Influence of Dietary Carbohydrate to Fat Ratio on Whole Body Nitrogen Retention and Hormone Response **41**

Introduction 41
Materials and Methods 42
Results 46
Discussion 55
References 62

4. Influence of Dietary Carbohydrate to Fat Ratio on Body Composition, Substrate Utilization, and Energy Expenditure in Healthy Male Subjects 65

Introduction 65
Materials and Methods 66
Results 69
Discussion 77
References 81

5. Influence of Dietary Carbohydrate to Fat Ratio on Whole Body Nitrogen Retention and Body Composition in Adult Rats 83

Introduction 83
Materials and Methods 84
Results 87
Discussion 92
References 97

6. Evaluation of Energy Metabolism of Rats fed Diets with High and Low Ratios of Carbohydrate to Fat 99

Introduction 99
Materials and Methods 101
Results 104
Discussion 111
References 115

7. Protein Synthesis Rates in Tissues of Rats fed Diets with High and Low Ratios of Carbohydrate to Fat 117

Introduction 117
Materials and Methods 118
Results 120
Discussion 126
References 131

8. Summary and Conclusions 133

Review of hypotheses 133
Future research 138
References 140

Appendices

Appendix I Experimental diets (human metabolic study)..... 141

Appendix II Changes in postprandial carbon dioxide production after diet treatment 144

Appendix III Calculation of heat production..... 145

Appendix IV Assay for the measurement of rates of protein synthesis..... 146

Appendix V Flooding dose techniques for measurement of protein synthesis: establishment of plateaus in specific radioactivity of intracellular fraction 148

Vita 149

List of Tables

3.1 Subject characteristics 42
3.2 Estimated diet composition 43
3.3 Protein and energy intakes 44
3.4 Total daily nitrogen excretion 47
3.5 Nitrogen balance of subjects on diet treatments 48
3.6 Fasting serum blood parameters 53
3.7 Postprandial serum blood parameters 54

4.1 Energy value of dietary intake and fecal losses during diet treatment 70
4.2 Anthropometric measurements after diet treatment 71
4.3 Resting energy expenditure during diet treatment 72
4.4 Changes in postprandial oxygen consumption after diet treatment 73
4.5 Non-protein respiratory quotients (NRPQ) and percentage of oxygen consumed by carbohydrate and fat 76

5.1 Experimental diets 85
5.2 Energy and protein intakes of rats fed diets providing different ratios of carbohydrate to fat 87
5.3 Nitrogen balance of rats fed diets providing different ratios of carbohydrate to fat 89
5.4 Body composition of rats fed diets providing different ratios of carbohydrate to fat 90

6.1 Composition of experimental diets 102
6.2 Gross energy intakes of rats fed diets providing different ratios of carbohydrate to fat 104
6.3. Energy losses in feces and urine of rats fed diets providing different ratios of carbohydrate to fat 105
6.4 Change in heat production after six weeks 108
6.5 Body weights of rats fed diets providing different ratios of carbohydrate to fat 109
6.6 Parameters of weight gain 109
6.7 Distribution of daily food energy 110

7.1 Protein and energy intakes of rats fed diets providing different ratios of carbohydrate to fat 120
7.2 Parameters of nitrogen metabolism 121
7.3 Body weights of rats fed diets providing different ratios of carbohydrate to fat 122
7.4 Tissue weights of rats fed diets providing different ratios of carbohydrate to fat 122
7.5 Specific radioactivity of intracellular phenylalanine in tissues of rats at different times after injection of L-[2,6 ^{3}H]-phenylalanine 123
7.6 Fractional rates of protein synthesis in tissues of rats fed diets providing different ratios of carbohydrate to fat 125

List of Figures

1.1 Model of protein metabolism....4
1.2 The urea cycle....8
1.3 Diet and hormonal factors affecting the distribution of body protein....22

3.1 Nitrogen balance during two diet treatments at maintenance energy intake....49
3.2 Nitrogen balance during two diet treatments at 75% of maintenance energy intake....50
3.3 Nitrogen balance and energy intake at maintenance energy intake....51
3.4 Nitrogen balance and energy intake at 75% of maintenance energy intake....52

4.1 Fasting and postprandial RQ at maintenance energy intake....74
4.2 Fasting and postprandial RQ at 75% of maintenance energy intake....75

5.1 Final body weights of rats fed diets providing different ratios of carbohydrate to fat for six weeks....88
5.2 Nitrogen balance per kcal energy gain in rats fed diets providing different ratios of carbohydrate to fat for six weeks....89
5.3 Percent of energy gain as protein calories or fat calories....91

6.1 Classification of energy in food....100
6.2 Twenty-four hour heat production (kcal/rat/hr) of rats fed diets providing different ratios of carbohydrate to fat....106
6.3 Twenty-four hour heat production (kcal/g/hr) of rats fed diets providing different ratios of carbohydrate to fat....107

7.1 Protein-bound specific radioactivity in tissues of rats fed diets providing different ratios of carbohydrate to fat....125

Chapter 1.
Literature Review

Introduction

The three macro-nutrients consumed by animals and man — carbohydrate, protein and fat — provide substrate for a wide range of metabolic functions. While all three nutrients yield energy to the body, fat and to a lesser extent carbohydrate are physiologically the most efficient sources (Donato, 1987). Protein is necessary for tissue growth and maintenance, but if total energy intake is inadequate, more protein is oxidized for energy.

The quantity and nature of the macro-nutrients absorbed has a major influence on the accretion of body protein. Dietary protein appears to stimulate both protein synthesis and degradation, whereas intake of non-protein energy, while affecting nitrogen retention, appears to act in a manner different from protein (Reeds and Fuller, 1983). These two factors — non-protein energy and protein energy — appear to have an additive rather than an interactive effect on protein deposition and may stimulate protein synthesis by different mechanisms.

For many decades, it has been widely accepted that total energy intake is a major factor involved in nitrogen balance, provided that protein intake is adequate (Young et al., 1981). Under such circumstances, if total energy intake is high, then nitrogen is retained. When low energy intake results in negative energy balance, nitrogen sources are mobilized from tissues. This is a direct interdependence which affects protein utilization.

The source of non-protein energy has been investigated to determine the physiological significance of the ratio of carbohydrate to fat in the human diet (Motil et al., 1981b; Richardson et al., 1979; Flatt, 1987). It has been suggested that carbohydrate alone exerts a specific action on protein

metabolism to decrease urinary nitrogen losses; fat does not have this effect (Munro, 1964). It is not known if there is an "ideal" ratio between the two nutrients that would promote protein-sparing and, although the literature favors a high carbohydrate intake, efforts to determine a mechanism have been inconclusive (Reeds and Garlick, 1984).

In animals, isocaloric intakes have resulted in higher body protein or fat gains, depending on diet composition (Hartsook et al., 1973; Toyomizu et al. 1982, 1985). Most evidence in animals is based on feeding trials during growth and there is no compelling evidence to suggest that inclusion of excess carbohydrate in diets leads to better utilization of protein for growth (Munro, 1964), unless the energy to protein ratio is not optimal. Adult humans and mature animals have a substantially different response to protein and energy intake by comparison with an infant or a depleted, injured or septic patient (Jeejeebhoy, 1981). Even the ability of young infants to store nitrogen is extremely limited compared to the growing rat (Hegsted and Neff, 1970). These factors limit the comparison of published reports because of inconsistencies in subjects and design.

Inefficient protein utilization implies numerous health concerns. This is especially true for populations where high quality dietary protein sources are limited. Appropriate intake of non-protein energy sources may improve bioavailability of the nitrogenous compounds. Various disease states and injury also lead to increased nitrogen losses (Askanazi et al., 1980; Walser, 1981; Waterlow, 1980). Diet composition may be an important factor in the efficacy of nutritional support.

With the advent of isotope technology, it is now possible to quantitatively assess metabolism of ingested protein under various conditions. It is important to determine underlying mechanisms for sparing of body protein because this

knowledge will help develop diets which are metabolically more economical for maintenance of adequate protein status and improvement of catabolic states.

For the purposes of the following review, the focus will be on nutrient utilization in a non-growth stage. The components of protein turnover and response of each component to diet composition will be discussed, followed by a review of indicators of protein-sparing. Possible mechanisms will be proposed.

Metabolic Aspects of Protein Turnover

The human body is composed of three main structural components — muscle, fat and bone. The muscle content is approximately 45% of total body weight in males and 35% of total body weight in females (Benke and Wilmore, 1974), and is greatly affected by protein status. Measurements of human cadavers have shown that protein content varies considerably among different body organs. Protein values have been estimated (g/kg) at 300 grams (skin), 192 grams (muscle), 163 grams (liver), 153 grams (kidney), 143 grams (heart) and 107 grams (brain) (Garrow, 1978). Thus, the fate of retained nitrogen may be in numerous locations in the body. Interpretation of measurements of whole body protein gain is improved if information can be obtained at the same time about rates of protein synthesis in different tissues (Waterlow, 1980). This is not feasible in humans. Changes that occur in whole body synthesis, degradation, and nitrogen balance represent the sum total of activity of various tissues and it remains uncertain if all tissues are affected equally. The rate of protein synthesis in skeletal muscle is believed to be more sensitive to nutritional change than other tissues (Reeds and Fuller, 1983; Rennie et al., 1982; Reeds and Garlick, 1984). Changes also occur in protein degradation, but these changes maximize short-term protein losses in the fasted state.

In the body, protein does not exist in a static state but is always in flux, reflecting the balance between protein synthesis, protein breakdown, and oxidation of amino acids (Figure 1.1). A healthy adult synthesizes and degrades 200 to 300 grams of tissue protein per day (Waterlow et al., 1978; Woolfson, 1983), even though usual intake may be one-third or less than this value. Small proportional changes in protein synthesis and degradation can produce marked changes in both nitrogen retention and free amino acid

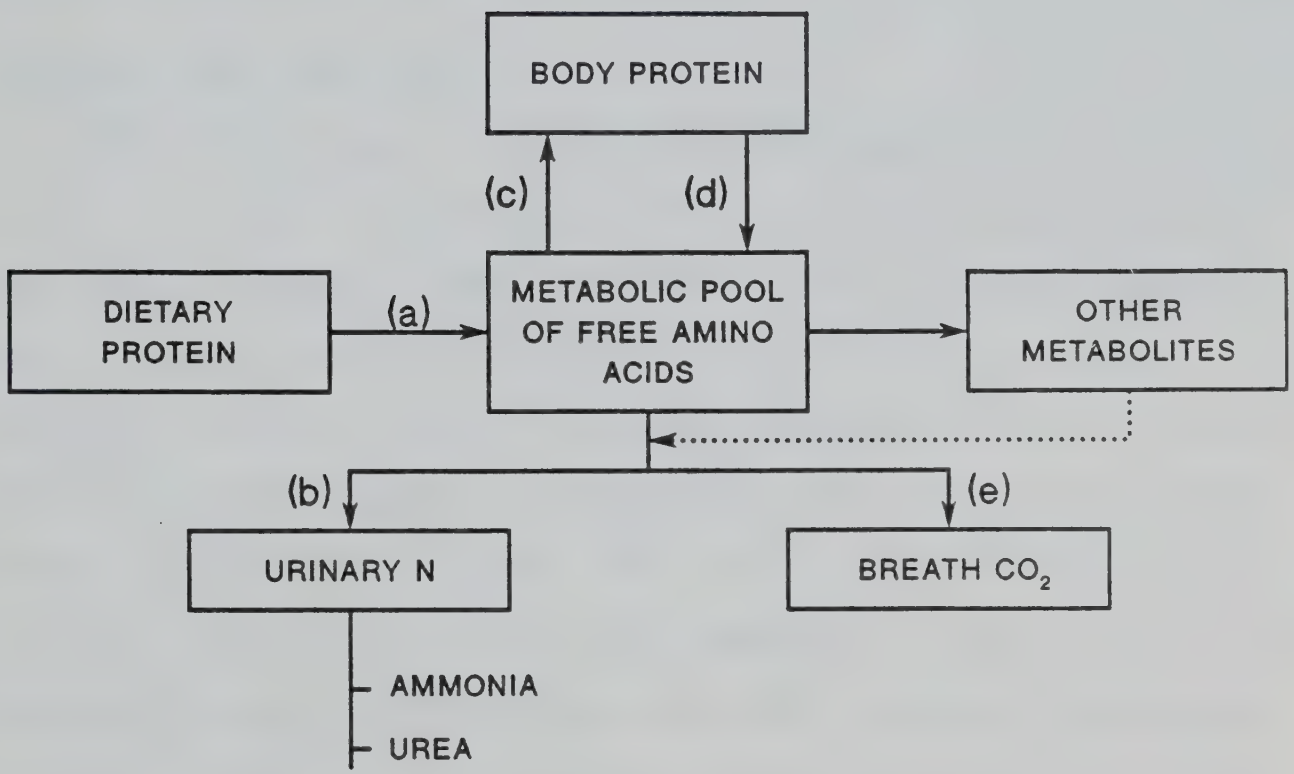

Figure 1.1 Model of protein metabolism
(Reeds and Garlick, 1984)
(a) digestion and absorption
(b) excretion
(c) synthesis
(d) degradation
(e) oxidation

concentrations (Reeds and Fuller, 1983). After a meal, positive nitrogen balance and expansion of the free amino acid pool occurs. The opposite occurs after a short fast (Reeds and Garlick, 1984). These changes are due to alterations in both protein synthesis and protein degradation.

In adults, body protein turnover is related to the maintenance requirement for diet protein, which remains relatively constant. It has been questioned if present methods are precise enough to yield accurate conclusions regarding the response of adults to changes in diet composition (Young et al., 1981). On the other hand, it is believed that provided the same methods are used, conclusions can be made regarding the direction of change, if not precise quantitative assessment.

Digestion and absorption

When dietary protein is consumed, digestion begins in the mouth as food is mixed with saliva. The process accelerates in the stomach as the protein is denatured by hydrochloric acid. High concentrations of proteolytic enzymes in the gut undergo slow autodegradation. Digestive enzymes secreted by the pancreas increase with higher concentrations of protein in the diet. Signals from the products of digestion may modify the pools of free amino acids in the tissues (Fauconneau and Michel, 1970). On a protein-free diet, there is a small fall in the concentration ratio (distribution in intracellular versus extracellular water) for most essential amino acids, except for lysine (which is reduced by 50%). The non-essential amino acids have much higher concentration ratios, believed to be due to formation from tricarboxylic acid cycle intermediates (Waterlow and Fern, 1981). There is no correlation between free and protein-bound essential amino acids. Thus, the free amino acid pool cannot be considered as a complete precursor pool. The amino acid

pool reflects changes in protein metabolism (i.e., synthesized protein and the products of its degradation) rather than causing changes in protein metabolism. Recent theories, however, suggest that certain amino acids have a regulatory function for protein synthesis and degradation. Changes in the relative rates of amino acid oxidation will also alter the pool (Waterlow and Fern, 1981). Some of the non-essential amino acids are made in cells (particularly muscle, liver, kidney, and brain cells) or through recycling (Waterlow et al., 1978).

Free amino acids and dipeptides, hydrolyzed in the intestinal epithelium, cross the intestinal wall. Absorption occurs primarily in the proximal jejunum, and then in the ileum (Silk, 1980). Peptides not utilized by intestinal tissue are carried by the portal circulation to the liver. The rate of absorption diminishes with the increased electric charge. Amino acids with non-polar side chains are rapidly absorbed (leucine, isoleucine, valine, tryptophan and phenylalanine), but glycine, aspartate, glutamate and arginine are slowly absorbed (Fauconneau and Michel, 1970). Munro (1964) suggested that the non-protein energy components of the diet affect protein absorption, and that it is at this step that differences in carbohydrate and fat intakes alter the nitrogen economy.

Unabsorbed dietary protein is excreted through the large intestine along with other nitrogenous compounds such as bacteria and desquamated epithelial cells. If 100 grams of protein are consumed in the diet, 70 grams of endogenous digestive proteins mix with food in the intestinal tract and approximately 160 grams of protein are then absorbed (Heymsfield et al., 1984a). Thus, fecal protein loss is about 10 grams per day, or 1 to 2 grams of nitrogen.

Excretion

Urinary nitrogen constitutes the largest source of nitrogen losses. When humans consume a mixed diet, urinary nitrogen is derived from urea (80%), ammonia (7.4%), creatinine (6.4%), uric acid (2-3%) and other minor compounds (1-2%) (Lentner, 1981). The amount of urea and ammonia varies, but the other components of nitrogen excretion remain relatively constant.

Ammonia is produced in the intestine and the kidney. It is readily diffusable to virtually all tissues, and the pH gradient across the renal cell determines its distribution between blood and urine. As a base, ammonia neutralizes acid derived from diet or body fluids and functions to preserve acid-base homeostasis (Alleyne et al., 1981). Metabolic alkalosis and acidosis respectively decrease and increase urinary ammonium excretion (Heymsfield et al., 1984a). If large acid loads are present, ammonia is generated from glutamine within the renal tubule cells and diffuses into the tubule to combine with protons:

$$NH_3 + H^+ \longrightarrow NH_4^+$$

The glutamine is produced and released by muscle and transported to the kidney, possibly at the expense of alanine. Thus, the excretion of muscle nitrogen switches from urea (a product of alanine metabolism in liver) to ammonia (a product of glutamine metabolism in kidney) (Newsholme and Leech, 1986; Cahill, 1970).

Metabolic alterations in serum amino acid concentrations and levels of urea cycle enzymes can affect ureagenesis. Urea is synthesized in liver (Figure 1.2) from its main precursors – alanine and glutamine (Felig, 1975). A portion of it (approximately 25%) is degraded in the bowel by bacterial urease to carbon dioxide and ammonia. The latter acts as a source of non-essential nitrogen and may be recycled via the liver for urea production (Walser and

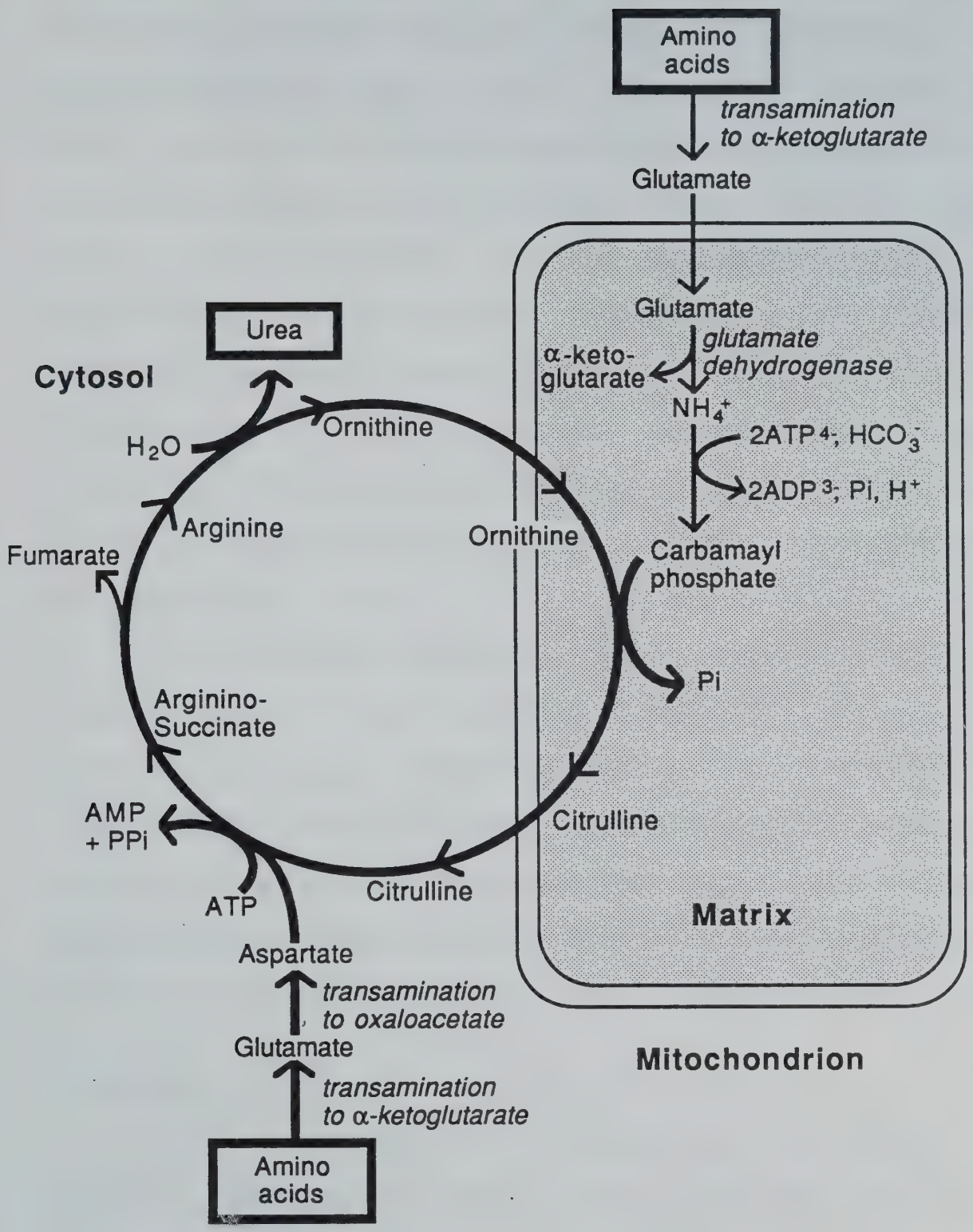

Figure 1.2 The Urea Cycle
(Lehninger, 1982)

Bodenlos, 1959). In hypermetabolic states (trauma, sepsis), excess alanine and glutamine are released from peripheral tissue. Initially, ureagenesis cannot keep up with the excess amino acids, which then build up in the blood along with ammonia (Walser, 1981). A key regulatory relationship has been shown where an increase in amino acid concentration produces a proportionately larger increase in the rate of ureagenesis. When carbohydrate intake is high, ureagenesis falls proportionately more than does the concentration of circulating amino acids (Walser, 1981). Walser (1981) suggested that progressive depletion of the amino acid pool may be minimized by carbohydrate feeding during periods of protein deprivation. Limitation by ureagenesis, however, is a rare occurrence. A high fat diet decreased urea synthesis with no change in circulating amino acid concentrations in growing pigs (Reeds et al., 1981).

Diet protein affects ureagenesis in the short term. It acts as a stimulus for N-acetyl glutamate, which activates mitochondrial carbamyl phosphate synthetase, a stimulus for ureagenesis (Tatibana et al., 1976; Hensgens et al., 1980). Over the long term, depending on the demands of the organism, protein in the diet induces enzymes of the urea cycle, a process which may take hours to days (Das and Waterlow, 1974). Both mechanisms function to maintain circulating amino acid concentrations within the optimal physiological range.

Synthesis

Protein synthesis occurs in the cell cytoplasm and involves ribosomes, messenger ribonucleic acid (RNA), transfer RNA, amino acids, adenosine-triphosphate (ATP), guanosinetriphosphate (GTP), and various enzymes (Pain, 1978). In animal cells, synthesis of all extramitochondrial RNA species takes place in the nucleus and these are transported to the cytoplasm for protein

synthesis (Dean, 1980). Regulation of protein synthesis can be exerted on one or more reactions common to synthesis of all proteins (translational control), rather than through availability of each messenger RNA. Even when new messenger RNA synthesis is prevented, total protein synthesis can change in response to nutritional or hormonal stimuli (Pain, 1978). Two sites of regulation have been suggested — the rate of protein synthesis per unit of ribosomes, also called ribosomal activity, and the ribosome content of cells and tissues. Both appear to be of importance in the nutritional and hormonal control of muscle protein mass.

The rate of body protein synthesis is much higher in premature babies and infants than in adults and elderly people. One comparison between age groups reports that, in grams of protein synthesized per kilogram body weight per day (g/kg/d), the premature infant averages 17.4 grams compared to the term infant (6.9 grams), the adult (3.0 grams) and the elderly individual (1.9 grams) (Young et al., 1975). These differences are essentially eliminated when protein synthesis is related to energy expenditure of different age groups. Basal metabolic rate and whole body protein synthesis are highly correlated (Reeds and Garlick, 1984; Waterlow, 1968; Young et al., 1975), and each are also correlated with estimates of dietary protein needs. It appears that the efficiency of dietary nitrogen utilization under optimum nutritional circumstances remains constant throughout the life cycle.

Energy is required for formation of peptide bonds in protein synthesis. This requirement has been estimated at ≤ 26% of total energy expenditure (Reeds et al., 1982), 15-25% of fasting metabolic rate (Waterlow et al., 1978), and 8-10% of fasting metabolic rate (Young et al., 1975; Kien et al., 1978). Although changes in the synthetic process alone can alter muscle mass, these are often accompanied by parallel increases or decreases in protein

degradation. The exception may be situations of severe depletion ("emergency situations") (Millward et al., 1976).

Body protein synthesis and amino acid oxidation decrease once food intake has ceased (Clugston and Garlick, 1982). In fasted man, decreases in skeletal muscle synthesis are greater (50%) than the decrease in whole body synthesis (35%) (Rennie et al., 1982). In the fed state, human muscle accounts for 53% of the whole body protein synthetic rate and it appears more sensitive than other tissues to feeding and fasting (Rennie et al., 1982).

Millward et al. (1976) completed extensive studies investigating protein synthesis in rat gastrocnemius and quadricep muscles, to assess breakdown and muscle loss after various diet treatments. Decreased synthesis but no change in breakdown resulted from a one-day protein-free diet, a ten-day low energy diet, and two days of glucocorticoid treatment. Large decreases in protein synthesis and breakdown occurred in rats that were diabetic, hypophysectomized, receiving a protein-free diet for nine and thirty days or starved for two days. A large decrease in synthesis with a large increase in breakdown occurred after four days of starvation in both 100 gram and 400 gram rats.

In skeletal muscle, changes in protein content and nitrogen retention can occur due to changes in protein synthesis rate (Millward et al., 1976; Reeds and Fuller, 1983). Dietary intake of protein and energy plays an important role. Insulin, growth hormone, and possibly glucocorticoids may exert controlling mechanisms. How protein breakdown rate is influenced is more uncertain (Reeds and Fuller, 1983).

Degradation

The rate at which a particular protein is degraded depends on the activity of the degrading system ("relative proteolytic environment") and the protein's susceptibility to the degrading system (Waterlow et al., 1978). With regard to the former, many proteins are much more stable in muscle than in liver, perhaps as a consequence of cell size. Susceptibility to a degrading system may depend on the protein's molecular weight, or conformational changes due to substrate binding and other structural changes (Waterlow et al., 1978). Increased rates of protein degradation may also occur due to increased number and/or activity of the lysosomes or to increased activity of non-lysosomal proteolytic enzymes (Mortimore and Schworer, 1980).

Protein breakdown decreases during protein deficiency (Millward et al., 1976). This decrease may be attributed to reduced triidothyronine (T_3) concentrations (Cox et al., 1981). Protein breakdown increases during fasting or starvation. This may be due to an increase in corticosteroids and decrease in insulin. Reduced insulin levels have been shown to increase degradation in vivo (Albertse, 1981) and in vitro (Jefferson et al., 1977). Hormones play a role in the control of protein degradation.

Oxidation

Under normal circumstances, 15% of resting energy expenditure results from oxidation of amino acids for energy in the liver and through branched chain amino acid oxidation in muscle (Woolfson, 1983). The amount of energy released is approximately 4.1 kilocalories for each gram of protein. This, however, varies somewhat depending on the amino acid pattern of the tissues.

Increases in protein intake above maintenance affect the rate of amino acid oxidation and have little effect on protein synthesis in adult humans (Motil

et al., 1981a) or in ruminants (Reeds et al., 1981). Similarly for individual amino acids, any excess above requirements is oxidized by the liver and the nitrogen is excreted as urea (Young and Bier, 1981; Krebs, 1972). Thus, the effects of non-protein energy sources on oxidation rates in adults must be studied at maintenance levels of protein intake (Richardson et al., 1979).

Protein-Sparing

Indicators of protein-sparing

The term "protein-sparing" has been defined as "reduction in excretion of nitrogen compounds in the urine" that occurs as a result of some treatment (Wolfe et al., 1977). In many circumstances, it is desirable that protein is spared — then amino acids are utilized for growth and/or tissue maintenance rather than for energy. Historically, indicators of protein-sparing have been the decreased output of urinary urea and ammonia with improvement in nitrogen balance. Urinary excretion of 3-methylhistidine has been used an indicator of contractile protein breakdown in skeletal muscle, but it also reflects non-utilizable methylhistidine released by other tissues (i.e., platelets, intestine) and thus it is not a good measure of muscle protein-sparing. Using stable isotope techniques, it is possible to investigate the status and integration of protein and amino acid metabolism within the body (Young and Bier, 1981). In animals, protein utilization can be measured by the incorporation of radioactively labelled amino acids into protein in tissues, organs, and the total carcass. Thus, changes in rates of protein synthesis and degradation have now become major indicators of protein-sparing.

Carbohydrate to fat ratio and protein-sparing: human and animal studies

In 1964, Munro asked whether carbohydrate and fat are equally effective as energy sources for promotion of nitrogen retention. This question is still not completely answered. On the basis of animal and human studies, it was thought that high carbohydrate intakes resulted in improved protein utililization because of three consistent findings:

1. Feeding carbohydrate (glucose) to fasting rats resulted in decreased urinary nitrogen output that was not observed with feeding fat (olive oil) (Munro et al., 1959). There was a significant loss, however, of liver nitrogen in the carbohydrate-fed group. Support for these observations came from a human study where fasted subjects, fed a high-carbohydrate diet, showed decreased urinary nitrogen (Hervey and McCance, 1952). The amount of carbohydrate necessary for this effect has been reported to vary between 100 grams (Gamble, 1946) and 300 grams per day (Wolthuis, 1961). Both urea nitrogen and ammonia nitrogen have shown decreases after carbohydrate administration.
2. Replacement of carbohydrate by fat at adequate protein and energy levels resulted in increased nitrogen output. A maximum effect occurred at 2 to 5 days, but a subsequent decline was observed after approximately 10 days. Even when only partial replacement with fat occurred, nitrogen losses increased, although the effect was less dramatic (Munro, 1964).
3. After carbohydrate feedings, plasma amino acids decreased within a few hours. When fat was fed, this decrease did not occur. Branched chain amino acids, which are predominantly affected by changes in diet (Adibi, 1976), have been shown to decrease in human plasma after glucose ingestion (Adibi et al., 1975). Increased plasma concentrations of branched

chain amino acids occurs in human subjects after consumption of a high fat diet at adequate protein intakes (Swenseid et al., 1967). This suggests increased gluconeogenesis or altered rates of protein synthesis and degradation. After a 24-hour glucose infusion in rats, plasma amino acids decreased with no increase in liver nitrogen (Munro et al., 1959). Muscle protein content increased instead.

It was concluded that after glucose ingestion or infusion, amino acids are deposited in muscle, depleting plasma amino acids in relative proportion to that required for net muscle protein synthesis (Munro and Thomson, 1953). When less amino acids are transported to the liver, urea production and excretion decreases.

Much of the earlier evidence for a protein-sparing effect of carbohydrate was observed in rats fed protein-free diets (Munro et al., 1959; Munro and Naismith, 1953; Rosenthal and Allison, 1956). If protein had been fed, the results may have been different. Other diets being fed were also nutritionally inadequate, which introduces other variables.

More recently, Richardson et al. (1979) fed diets of different carbohydrate to fat ratios (1:1, 2:1) to healthy male subjects. Protein intake was set at 0.6 g/kg/d, considered a safe level of intake by FAO/WHO (1973). Significant decreases in fasting serum urea nitrogen, total urinary nitrogen, and urinary urea nitrogen occurred when subjects were fed the high carbohydrate diet (carbohydrate – 62%; fat – 31% of total energy). The high carbohydrate to fat ratio resulted in improved nitrogen balance and protein utilization compared to the diet of equal fat and carbohydrate content (47% of energy). This was only true at intakes of less than 50 kcal/kg, and above this level the opposite was observed. The protein-sparing effect appeared to be greatest in subjects who lost weight, suggesting that either protein or energy intakes were marginal.

Richardson et al. (1979) did not observe a decline in nitrogen losses after 10 days on the lower carbohydrate diet as reported by Munro (1964). Subjects were fed each diet for 21 days, representative of more long-term effects.

Motil et al. (1981b) used isotope tracers of 1-^{13}C-leucine and ^{15}N-lysine to study effects of excess intake (25% above maintenance) of different energy sources on protein turnover. Diets fed provided 0.6 g/kg/d of protein. One diet was approximately 60% carbohydrate, 5% protein and 35% fat. The second diet was 40% carbohydrate, 5% protein and 55% fat. Estimates of leucine retention were significantly greater with diets containing higher levels of carbohydrate. This was manifested by decreased oxidation, and slightly increased synthesis and breakdown of the amino acid. More human studies are necessary to confirm these results. In these two previous studies, energy intakes were slightly higher on the high-carbohydrate diets.

Man is not unusual from other species in his rate of protein synthesis or its relationship to energy expenditure. Generally, information from animal studies may be cautiously inferred to human application (Reeds and Garlick, 1984). Effects of diet composition on protein utilization were examined in rats (Hartsook et al., 1973) and chickens (Toyomizu et al., 1982, 1985) when protein intakes and carbohydrate to fat ratios varied over a wide range. Dietary energy was held constant. These studies were of young, growing animals and as such, protein intakes exerted the greatest effect on growth, body protein gain, and body energy gain. In rats at intakes of 22% protein, nitrogen gain was greater on a high fat diet. A high carbohydrate to fat ratio increased efficiency of nitrogen gain, but only when protein intake was above 44% of total energy. At lower protein intakes (< 36%), a high carbohydrate intake increased digestible and metabolizible energy and increased heat production; fecal and urinary nitrogen, however, also increased (Hartsook et al., 1973). In

chickens, there was no effect of the carbohydrate to fat ratio on protein gain and minimal effect on metabolizible energy. Energy gain increased as carbohydrate to fat ratio decreased (Toyomizu et al., 1982, 1985).

Carbohydrate to fat ratio and protein-sparing: short term effects of meal feeding

Munro (1964) suggested that carbohydrate affects absorption of protein-containing meals, and that the beneficial effect on nitrogen retention appears to be lost if carbohydrate is fed more than four hours after protein feeding. Similarly, with infusates, it is more beneficial if glucose is given with amino acids than at separate times (Sim et al., 1979). This point is of more significance to intravenous feedings, because most human diets include mixed meals, not intakes of single nutrients.

Motil et al. (1981a) examined response to protein in fed versus fasted states in healthy males at three levels of protein intake. At maintenance protein intakes (0.6 g/kg/d), protein breakdown decreased with meal feeding. This response was greater with an abundant protein intake. There was no change in oxidation or synthesis. It is believed that the rate of flow of endogenous amino acids into the labile pool decreases with meal feeding. Conway et al. (1980) fed diets adequate in protein and energy to healthy male subjects. Decreased protein breakdown was also seen after meals. When lysine was removed, whole body protein breakdown was still decreased with meal-feeding compared to the post-absorptive state. It is possible that only certain amino acids, or the source of energy, stimulate changes in rates of protein breakdown after meals.

With obese female subjects, Garlick et al. (1980a) found results on meal-feeding response contrary to those of the previous authors (Motil et al., 1981a;

Conway et al., 1980). Rates of whole body synthesis and leucine oxidation during the night (fasted state), were 67% and 38%, respectively, of rates during the day when small meals were given hourly. Whole body protein breakdown increased slightly with fasting. Dissimilar results were attributed to differences in meal size and meal composition of the two studies rather than to the obesity, because controls responded the same way. Young et al. (1981) carried out a paired study which gives qualitatively similiar results to Garlick et al. (1980a).

Many physiological and biochemical parameters show diurnal rhythms. Garlick et al. (1980a) attributed higher rates of protein synthesis and oxidation during the day to food intake. It is essential that in studies of protein metabolism, meal composition and timing is strictly controlled due to reported variations in response to meal-feeding.

Carbohydrate to fat ratio and protein sparing: enteral and parenteral feedings

Extensive work on the source of non-protein energy substrates and protein utilization has been done with intravenous feedings, removing the variables of digestion and absorption. Sim et al. (1979) investigated effects of oral feedings compared to intravenous amino acids, and amino acids plus glucose in five healthy male subjects. The isonitrogenous, isoenergetic infused diet resulted in a slightly negative nitrogen balance as well as decreased protein synthesis, degradation and turnover compared to the oral diet. Higher insulin levels with the intravenous solution were observed. Protein degradation was slightly greater than protein synthesis, although no muscle loss was apparent. It was proposed that the decreased need for secreted proteins in the gut resulted in the decrease in protein turnover observed with the infusate. Total protein secretions in the gastrointestinal tract

can be substantial (Waterlow, 1978). Amino acids taken by mouth may also be utilized differently from those given intravenously (Bloom, 1978). No difference in nitrogen balance was observed in humans (Patel et al., 1973) or in pigs (Keith et al., 1975) when similar intravenous and enteral diets were fed. However, total protein turnover may have been altered with similar changes in protein synthesis and degradation, but no net change in balance.

No clear answer is available as to the preferred non-protein energy source for intravenous feedings. Hospitalized patients have primarily been the subjects of these investigations, which introduces the variables of an altered somatic status and endocrine response. Variability of acutely ill or depleted patients with respect to nitrogen balance is great (Nordenstrom et al., 1983).

Long et al. (1977) have suggested that in the stressed condition, the amount of carbohydrate infused is the major substrate which determines nitrogen retention. In acute studies (3 days) of stable post-operative or post-injury patients, urea nitrogen excretion and carbohydrate intake were inversely related (Long et al., 1977). Woolfson et al. (1979) have demonstrated that glucose alone is marginally more protein-sparing than fat-based total parenteral nutrition. Other studies have suggested that carbohydrate is more effective than lipid in conserving nitrogen (Shizgal and Forse, 1980, 1981; Spanier and Shizgal, 1977; Tulikoura and Huikuri, 1981).

In sixteen gastrointestinal patients given total parenteral nutrition (TPN) for two weeks (carbohydrate and amino acids versus carbohydrate and amino acids and 60% of energy as fat), the addition of fat to the emulsion resulted in no significant water or fat gain, but rather a gain in protein. The glucose solution led to gains of water and fat, but not protein (Macfie et al., 1981). In one patient receiving long-term parenteral nutrition, available evidence shows that the use of fat is associated with body protein repletion (Jeejeebhoy et al.,

1973a). The solution was provided at a carbohydrate to fat ratio of approximately 1:1, for the first five months, but then changed to high carbohydrate feedings. Also, "it has been shown that intravenous fat exerts a nitrogen-sparing effect in adults in certain hypercatabolic states and in children" (Beisbarth, 1976). An emulsion of amino acids and fat more adequately maintained albumin synthesis than amino acids and glucose in rhesus monkeys (Wannemacher et al., 1978).

Many investigations have found both non-protein energy sources to be equally effective in promoting nitrogen retention. This has been shown in cases of malnutrition (Gazzaniga et al., 1975), in the presence of sepsis (Wannemacher et al., 1978), and in gastrointestinal patients (Jeejeebhoy et al., 1976).

More recently, fat- or carbohydrate-based total parenteral nutrition resulted in similar increases in body weight, nitrogen balance, muscle protein and liver protein content in rats (Boelhouwer et al., 1983), and in humans, no differences were observed in whole body protein synthesis, breakdown, or net synthesis (Baker et al., 1984). No differences in nitrogen balance were seen when fat- or carbohydrate-based enteral feedings were given to patients (Heymsfield et al., 1984b) or when TPN was given to acutely ill or depleted patients (Nordenstrom et al., 1983). Nordenstrom et al. (1983) suggested that carbohydrate influences protein-sparing only at low intakes (100-150 grams) and that after obligatory glucose requirements are met (2-4 g/kg/d, or 20-40% of total energy), the effects of fat and carbohydrate are equal. There may be a maximal rate of glucose infusion beyond which physiologically significant increases in protein synthesis and direct oxidation of glucose cannot be expected (Burke et al., 1979).

A Mechanism for Protein-Sparing

The response of protein turnover to nutrient intake appears to be due to two possible mechanisms: the indirect effect of hormones, or the direct effect of substrates, their precursors and end-products (Reeds and Fuller, 1983). The two may not be separate, because control may be initiated as a result of the stimulus to hormone secretions provided by absorbed nutrients.

Hormonal effects

Hormones are influenced by dietary intake. Various hormones influence protein synthesis. It has been proposed that a hormonal link is involved in protein-sparing. Reeds and Fuller (1983) suggest investigation of insulin, cortisol, growth hormone, and glucagon, with regard accorded to the balance between anabolic and catabolic factors. Thyroid hormones and androgens and estrogens also have a significant role in protein deposition (Buttery, 1983). Insulin, growth hormone and androgens increase muscle protein synthesis and decrease degradation. Corticosteroids, thyroid hormones and glucagon decrease muscle protein synthesis and increase degradation. Hormonal effects on muscle protein mass are usually paralleled by similar changes in nitrogen balance, and opposite changes in visceral proteins (Figure 1.3).

Insulin

Insulin is secreted by the pancreas and is anabolic for protein metabolism. It stimulates amino acid transport and synthesis of protein, RNA and DNA (Buttery, 1983). Insulin stimulates the uptake of amino acids by peripheral tissues rather than by liver (Brockman et al., 1975). It clearly favors anabolism, glucose oxidation, and inhibition of lipolysis (Brennan et al., 1975). Glucose and insulin yield considerable improvement in nitrogen balance compared to glucose alone or fat emulsion (Woolfson et al., 1979).

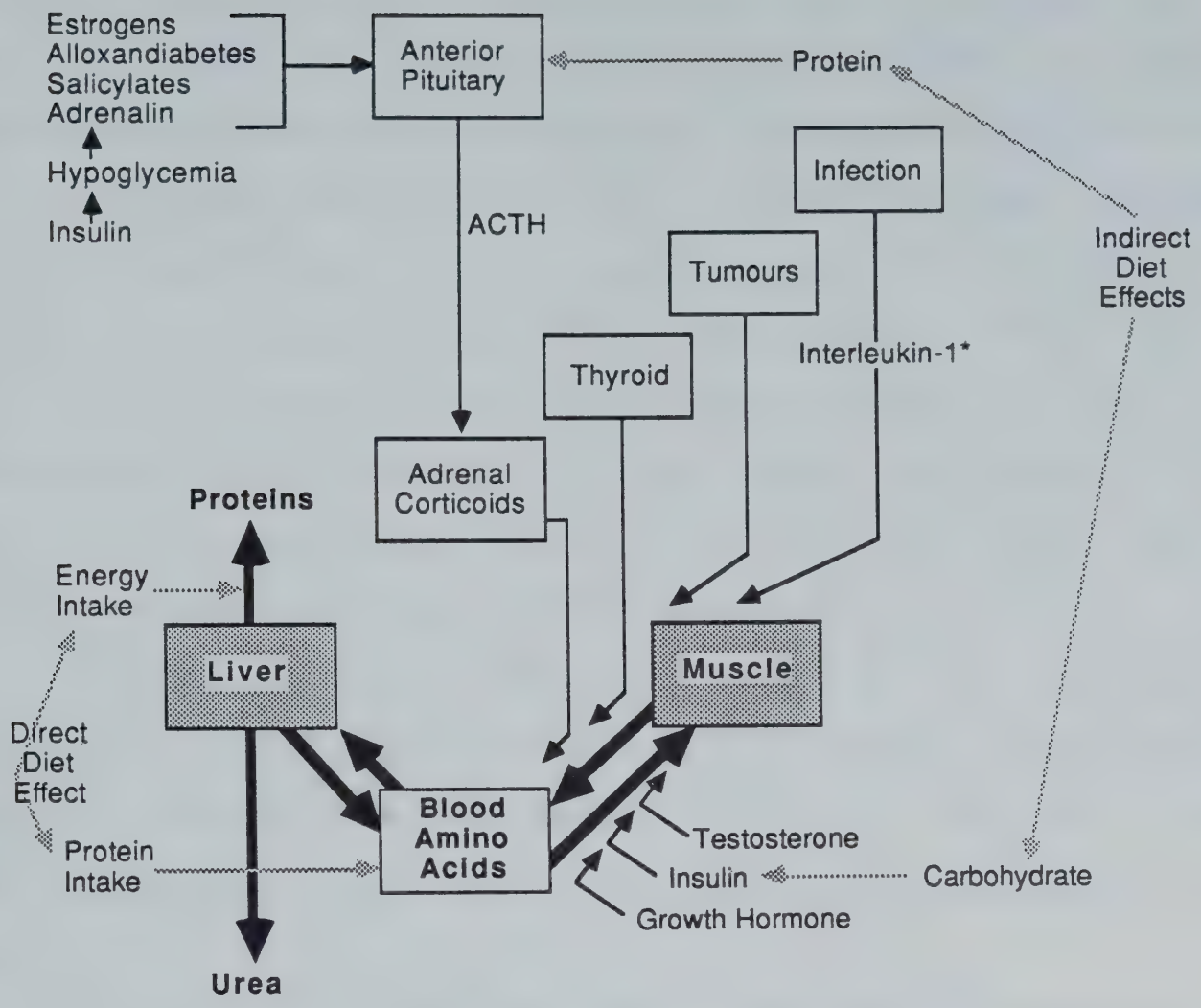

Figure 1.3 Diet and hormonal factors affecting the distribution of body protein
(Adapted from Munro, 1964)
* Not included in original figure

Munro (1964) first postulated that the mechanism for protein-sparing with increased carbohydrate intake was due to insulin release, which caused amino acid deposition in muscle. This theory was supported by various animal experiments. Pancreatectomized dogs on a high carbohydrate diet showed decreased urinary nitrogen only after insulin was re-administered (Chaikoff and Forker, 1950). Fuller et al. (1977) infused insulin and glucose at levels to prevent hypoglycemia in healthy pigs. They observed an increase in plasma

insulin and large decreases in plasma urea and urinary urea nitrogen, which returned to normal when the infusion was stopped. Millward et al. (1976) reported low synthetic activity in diabetic rats. In isotope studies, pigs were fed a high carbohydrate diet and infused with ^{14}C-urea and ^{3}H-leucine. Plasma insulin increased transiently, cortisol decreased, and amino acid catabolism decreased with a reduction in urea formation (Reeds and Fuller, 1983). In burn patients, insulin infusion reduced nitrogen excretion by 20%, but only when pre-treatment nitrogen loss exceeded 15 grams per day. This amount is rarely seen in any other type of patient (Woolfson et al., 1979). The retained nitrogen is believed to reflect an increase in protein synthesis (Burke et al., 1979).

Wolfe et al. (1977) stated that "no single endocrine set is absolutely essential for improved nitrogen economy." Efficient utilization of infused amino acids was achieved at both low ketone and elevated insulin levels (high carbohydrate diet) and high ketone and unchanged insulin levels (high fat diet).

It has been questioned if the effect of carbohydrate is independent of insulin. Glucose resulted in decreased urea formation in perfused rat liver (Miller, 1961) and diabetic rats showed nitrogen-sparing when given glucose, without insulin (Bancroft et al., 1951). Recently, Buttery (1983) suggested that the growth-promoting effects of insulin (i.e., DNA synthesis) may be mediated by pathways distinct from its effects on carbohydrate metabolism. The amount of insulin necessary for maximal protein utilization is small. Normally, it may be as little as that observed in the fasting state (Jeejeehboy, 1981). In the study of Richardson et al. (1979), which showed protein-sparing with high carbohydrate intakes, serum insulin and glucose levels were similar between the two diet periods.

Growth hormone

Growth hormone is also anabolic for protein synthesis. It has a similar effect to insulin in that it increases amino acid incorporation into muscle protein and decreases plasma amino acids and liver protein content (Eisenstein and Singh, 1980). Total nitrogen retention improves with growth hormone administration to normal and hypophysectomized rats, with subsequent increases in body weight and body protein. It also promotes lipid mobilization and oxidation. Protein synthesis is decreased to low levels in hypophysectomized rats (Millward et al., 1976). Growth hormone may exert its effects indirectly through the action of somatomedins. These are peptide hormones which are not stored, but are produced in response to growth hormone, either in liver or in target tissue which may mediate protein synthesis. In many bioassays, insulin and somatomedins yield similar results (Hall and Luft, 1974).

Androgens

Androgens have a similar effect to that of insulin and growth homone on nitrogen retention (Munro, 1964). Testosterone is anabolic for muscle protein synthesis (Kochakin, 1975). Increases in both growth rate and muscle mass have been seen with androgen administration. Diet appears to have no direct effect on androgen stimulation.

Corticosteroids (Glucocorticoids)

Corticosteroids (cortisone, cortisol) secreted from the adrenal gland are important for mobilization of protein stores. It is believed that the catabolic effect on muscle protein is due to suppression of protein synthesis rather than an increase in protein breakdown (Waterlow et al., 1978; Young et al., 1971). In rats treated with corticosteroids, there was decreased uptake of labelled

amino acid into muscle protein in vivo (Hanoune et al., 1972) and glucocorticoids suppress muscle protein synthesis in vitro (McGrath and Goldspink, 1982; Reeds and Palmer, 1983). Plasma amino acids increase and plasma liver protein synthesis increases after cortisol treatment. Plasma protein synthesis increases along with increased plasma amino acid levels, proportional to plasma leucine levels (Jeejeebhoy et al., 1973b).

The dose of corticosteroids may be critical in understanding their physiological significance. No change in protein metabolism was observed in adrenalectomized rats given physiological doses (Tomas et al., 1979). A pharmacological dose given to rhesus monkeys resulted in increased plasma amino acids, insulin levels, muscle breakdown and decreased growth (Wannemacher et al., 1978).

Corticosteroids can induce hyperinsulinemia; thus, changes in protein turnover are not as dramatic as would be expected from a catabolic hormone.

Cortisol activity is under some control by the anterior pituitary, which may provide a stimulus in response to dietary protein, estrogens, adrenalin or hypoglycemia (Munro, 1964). A high carbohydrate intake, and subsequent increased insulin levels, inhibits corticosteroid release, and as such, protein would be spared indirectly.

Glucagon

Glucagon is secreted by the pancreas in response to low blood sugar levels. It stimulates catabolism of protein by the liver for gluconeogenesis, an action which is prevented by insulin. In this respect, a high carbohydrate diet would prevent hepatic protein breakdown when it stimulates insulin secretion. Increased glucagon levels would not be inhibited with a high fat diet, because

insulin would not be stimulated. When the insulin to glucagon ratio is high, nitrogen economy is most readily favored (Wolfe et al., 1977).

Thyroxine

Thyroxine (T_4) is secreted by the thyroid gland and converted to 3-5-3' triiodothyronine (T_3), or to 3-3-5' triiodothyronine (rT_3) in liver and kidney. Hypothyroidism is associated with reduced rates of protein synthesis, as is hyperthyroidism (Munro, 1964). However, increased synthesis has also been shown with hyperthyroidism (Brown et al., 1981; Nair et al., 1981). Thus, a physiological range must be determined or results will conflict. It appears that T_3 increases incorporation of amino acids into protein and possibly stimulates RNA formation (Eisenstein and Singh, 1980). In rat liver, it was found that T_3 administration increased messenger RNA for at least three proteins (growth hormone, $_{2u}$-globulin, and malic enzyme) (Liaw et al., 1983). Protein synthesis and oxygen consumption increased in both thyroidectomized and hypophysectomized rats after T_3 administration (Millward et al., 1979). Thyroxine (T_4) results in loss of protein from peripheral protein stores, increases in liver protein (Eisenstein and Singh, 1980) and increases in protein synthesis in rat heart (Hjalmarson et al., 1975).

Diet composition may have independent effects on thyroid hormone metabolism. A high-carbohydrate low-protein diet results in elevated T_3 and decreased rT_3 levels in animals (Edozien et al., 1978; Burman et al., 1979) and in man (Danforth et al., 1977; Spaulding et al., 1976). Fasting leads to opposite effects on T_3 (decrease) and rT_3 (increase) blood levels, but no change occurs in serum T_4. Normal values will return with feeding of a high carbohydrate or mixed diet, but not with a high fat diet (Danforth et al., 1981). Increased energy intake also elevates T_3 levels. Carbohydrate influences T_3 and rT_3, especially

when energy intake is equal to or slightly less than maintenance. Therefore, if energy intake is kept constant, the diet composition may play a central role in thyroid metabolism.

Substrate effects

The different substrates may initially exert some effect which alters protein metabolism. Most studies have focused on nitrogen utilization rather than energy utilization, but diet composition also influences metabolizable energy, energy expenditure, and thus energy efficiency.

Metabolizable energy is the energy directly available to the animal (gross energy – [fecal energy + urinary energy]) (Leveille and Cloutier, 1987). In terms of the original Atwater factors, metabolizable energy, as a percentage of gross energy, is higher for carbohydrate (96.4%) than for fat (94.7%) (Maynard, 1944). In growing rats, metabolizable energy decreased as fat in the diet increased (Hartsook et al., 1973). This supply of energy is transformed into heat and net energy. When more heat is produced, this results in less energy available for work and tissue storage (Leveille and Cloutier, 1987). Diet composition may affect these energy components.

There is an interaction between protein synthesis and energy expenditure. Resting metabolic rate and protein turnover have been measured in man under various conditions. When resting metabolic rate increased by at least 10% during periods of thyrotoxicosis (Nair et al., 1981), growth (Golden et al., 1977) and after vaccination (Garlick et al., 1980b), protein synthesis increased by 39%, 92%, and 37% respectively. In situations where resting metabolic rate decreased by at least 10% with underfeeding (Nair et al., 1981) and aging (Golden and Waterlow, 1977), protein synthesis also decreased. The rate of protein synthesis is an important factor in determining energy

expenditure (Garrow, 1981), and both will generally change in the same direction in response to nutrient intake (Waterlow, 1968; Garrow, 1981).

Long et al. (1977), using infusions of different nutrient composition, showed a direct relationship between carbohydrate intake and metabolic rate. This was also observed by Baker et al. (1984) in gastrointestinal patients, and by Heymsfield et al. (1984b) when enteral feedings were administered. However, no differences in resting energy expenditure were observed when glucose or glucose and lipid (50/50) were infused (Nordenstrom, 1983). The slope of heat production versus energy infusion rate of enteral feedings (energy produced/energy increase in dose) resulted in higher values when a high fat diet (83%), compared to a high carbohydrate diet (69%), was infused (Heymsfield et al., 1984b).

When lipid was added to a TPN solution, it was effectively used as a fuel source, shown by a fall in respiratory quotient (RQ) (Baker et al., 1984). In animal studies, RQs shifted downward during fat infusion to reflect fat utilization (Geyer, 1970). Based on oral intakes, Flatt (1987) has shown a prior diet effect on RQ values. When protein is constant, a diet with carbohydrate levels of 80% (fed for 6 days), 60% (3 days), and 14% of energy (3 days) resulted in fasting non-protein RQs of 0.93, 0.82 and 0.76 respectively. Five hundred grams of dextrin-maltose were fed to subjects on the three diets. In a high carbohydrate diet, the glucose is used for glycogenesis first, then lipogenesis. After 15 hours, the cumulative fat synthesis and fat balance was highest with the high carbohydrate diet and lowest with the high fat diet. With the latter diet, there was increased fat oxidation. It is suggested that the addition of fat may enable more energy to be oxidized along alternate pathways, resulting in more efficient use of the administered energy, and leaving more available for net protein synthesis (Macfie et al., 1981).

When equal amounts of food energy, differing only in the proportion of carbohydrate and fat, are provided to animals and man the metabolic response is different. In a number of complex events, differences occur in endocrine response and energetic efficiency. Some control is exerted on the intake, storage and disposal of each of the macronutrients and it is believed that the balance of each one is regulated separately (Bray, 1987). Although the main focus of this research is on protein balance, the components of carbohydrate and fat balance must also be considered.

Summary

Although a protein-sparing effect of high carbohydrate to fat ratios has been most widely reported, the phenomenon has not been a consistent finding. Furthermore, no clear mechanism has been found. What little evidence there is suggests that a small increase in body protein synthesis and a small decrease in degradation underlie the response to carbohydrate supplementation. The effects of protein-sparing may be a direct consequence of the major substrate and alterations in energy utilization, or of subtle hormonal changes affecting protein metabolism. Studies of hospitalized patients receiving intravenous solutions of different energy sources have been inconclusive. While there are studies in control subjects, these have not been supported by measurements of energy metabolism, and only Motil et al. (1981b) investigated leucine metabolism. Controlled studies in healthy subjects and animal models are necessary to assess whole body energetics and the endocrine response which coincides with changes in nitrogen retention due to diet composition. When these metabolic changes are identified in the normal subject, this information will enable more consistent and effective nutritional support for various disease states, to optimize maintenance of lean body mass.

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Chapter 2.
Research Plan

Rationale

Protein is an essential nutrient. There are nine amino acids needed for protein synthesis which cannot be made by the body. Thus, some protein must be consumed in the diet. In some parts of the world, protein deficiency is common and good quality protein sources are scarce. In certain disease states, where appetite and food intake may be poor, nitrogen losses are high. In such cases, it is important that the protein consumed is maximally utilized for body tissue growth and maintenance.

It is believed that the source and quantity of dietary energy is related to protein utilization. At sub-maintenance energy intakes, amino acids are required for glucogenic processes. This decreases the availability of substrate for protein synthesis. At maintenance energy intake, a high proportion of carbohydrate in the diet has been shown to increase nitrogen retention in animals and man, but this remains controversial. A high proportion of fat in the diet appears to yield greater energy deposition.

With changes in diet composition, no precise protein-sparing mechanism has been determined. Knowledge of this mechanism would aid in the development of nutritional support for conditions of protein deficiency, trauma, sepsis, burns, and other disease states, resulting in predictable whole body responses to diet and preservation of the patient's nitrogen economy.

Hypotheses

1. Whole body nitrogen retention is influenced by diet composition. When the non-protein energy component of the diet is supplied as a low carbohydrate to fat ratio (high fat diet) versus a high carbohydrate to fat ratio (high carbohydrate diet), protein-sparing is enhanced.

2. Alterations in nitrogen retention occur as a result of changes in available energy due to diet composition. When the non-protein energy component of the diet is supplied as a low carbohydrate to fat ratio versus high —
   (a) energy expenditure decreases
   (b) fat oxidation increases
   (c) fat deposition increases
   (d) heat loss decreases.

3. Alterations in nitrogen retention occur as a result of hormonal changes due to diet composition. When the non-protein energy component of the diet is supplied as a low carbohydrate to fat ratio versus high, the balance of anabolic and catabolic hormones favors protein synthesis.

4. Increased net nitrogen retention occurs as a result of increased protein synthesis.

Objectives

Hypotheses will be tested in human and animal experiments.

Hypothesis #1:

1. Nitrogen retention, determined by nitrogen balance, will be measured in healthy male subjects. Diets composed of normal foods providing a

carbohydrate to fat ratio of 1:1 versus 2:1 will be consumed. Nitrogen balance will be tested at maintenance and 75% maintenance energy intakes to assess the influence of energy balance (Chapter 3).

2. Nitrogen retention, determined by the nitrogen balance method, will be measured in adult rats. Six nutritionally balanced diets with carbohydrate to fat ratios of 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, and 3:1 will be provided in isocaloric, isonitrogenous amounts for six weeks. The wider range of macronutrient ratios will be used to test for a linear response. Carcass protein content will also be measured (Chapter 5).

Hypothesis #2:

1. Energy expenditure and fat oxidation will be assessed by indirect calorimetry. Body fat composition and body weight change will be assessed by anthropometric measurements in healthy adult subjects fed diets providing a carbohydrate to fat ratio of 1:1 versus 2:1 (Chapter 4).

2. Carcass fat content of adult rats fed diets with carbohydrate to fat ratios of 0.5:1 – 3:1 will be measured (Chapter 5).

3. Energy retention in adult rats will be determined by energy balance techniques. Measurements of:
   - energy expenditure
   - fat oxidation
   - heat loss
   - carcass energy

   will be done for animals fed diets of either a high or low carbohydrate to fat ratio (Chapter 6).

Hypothesis #3:

1. Serum levels of insulin, growth hormone, cortisol and triiodothyronine will be measured in healthy male subjects fed diets providing a carbohydrate to fat ratio of 1:1 versus 2:1. These will be measured in the fasted and fed states to assess the acute effects of diet composition (Chapter 3).

Hypothesis #4:

1. Protein synthesis in tissues of adult rats will be measured by the incorporation of ^{3}H-phenylalanine into liver, muscle and intestine. Animals will be fed diets of either a high or low carbohydrate to fat ratio (Chapter 7).

Chapter 3.
Influence of Dietary Carbohydrate to Fat Ratio on Whole Body Nitrogen Retention and Hormone Response

Introduction

The nutrient composition of the non-protein energy in the diet may have some influence on nitrogen balance. Richardson et al. (1979) found that the most efficient nitrogen utilization was achieved when carbohydrate was the major energy source, rather than fat, in a liquid diet fed to healthy male subjects. This was the case at energy intakes of less than 50 kcal/kg, but the opposite was observed at higher intakes. More recent studies have shown that increased fat in the diet yields a beneficial effect on nitrogen metabolism (Nose et al., 1985; Pineault et al., 1985). The effect was seen in intravenously-fed infants. The literature is extensive and controversial on this point. Much of the research has been done on hospitalized patients. In unhealthy subjects, there are many disease-related variables that may confound any effect of diet composition. Also, no clear mechanism has been established which links the composition of non-protein energy to nitrogen retention.

The present study was designed to examine the effect of altering the level of carbohydrate to fat in the diet (2:1 versus 1:1) on nitrogen retention in healthy subjects. Comparisons were made at maintenance and 75% maintenance energy intake levels to assess the influence of energy balance. Blood levels of energy substrates and hormones involved in protein turnover were measured to assess possible mediators of a protein-sparing mechanism.

* A combined version of Chapters 3 and 4 has been accepted for publication:

McCargar L.J., Clandinin, M.T., Belcastro, A.N. and Walker, K. Influence of dietary carbohydrate to fat ratio on whole body nitrogen retention, substrate utilization and hormone response in healthy male subjects (accepted, Am. J. Clin Nutr. 1988).

Materials and Methods

Subjects and experimental design

The subjects were six young male university students (Table 3.1). Criteria for participation in the study required sedentary, non-smoking subjects, free from chronic illness, having no requirement for medication, and weighing less than 77.0 kg with a body fat content between 10 and 20%. Initial assessment included a medical history, lifestyle questionnaire, diet and activity history and anthropometric measurements (height, weight, skinfold measurements to assess percentage body fat) (Durnin and Womersley, 1974). The purpose and requirements of the study were fully explained to each subject before they gave informed consent. The study was approved by the Ethics Review Committee for Human Experimentation, Faculty of Medicine, University of Alberta.

Table 3.1
Subject characteristics*

Subject Initials	Age *yr*	Height *cm*	Body Weight *kg*	Ideal Body Weight %	Body Fat %
KC	22	176	71.5	94.5	17.3
PL	24	172	61.0	91.3	13.8
JX	29	169	53.7	86.9	12.0
HB	28	174	61.8	96.9	19.8
NT	19	182	76.5	102.7	21.2
JE	29	182	72.3	90.4	13.0
X ± SEM	25 ± 2	176 ± 2	66.1 ± 3.6	93.8 ± 2.3	16.2 ± 1.6

*Determined at the beginning of the first diet treatment.

During the study, subjects followed their usual routine, except they reported daily to the metabolic laboratory to be weighed and to eat breakfast (8:00 a.m.) and lunch (12:00 p.m.). A pre-packaged supper and snack were provided to be taken with them. The four daily meals were isocaloric and isonitrogenous. Subjects were instructed to eat their meals at the same times daily (approximately 4-5 hours apart). All subjects were maintained on the same feeding and testing schedule to ensure similarity in the response of body protein metabolism to food intake.

Each subject received four different diet treatments. The non-protein energy was supplied at two ratios of carbohydrate and fat (2:1 and 1:1) randomly assigned, and at two energy levels (maintenance and 75% maintenance requirements) (Table 3.2). Energy requirement was determined using the Mayo Clinic Nomogram (Food Nomogram, 1981) by a method

Table 3.2
Estimated diet composition*

Cho:Fat Ratio	Protein		Carbohydrate		Fat		Fiber‡ (crude)	P:S‡
	g	*(%)*	*g*	*(%)*	*g*	*(%)*	*g*	Ratio
Maintenance Energy								
High Carbohydrate (2:1)†	56	(7)	479	(62)	107	(31)	7.1	1.1
High Fat (1:1)	56	(7)	360	(46.5)	160	(46.5)	6.3	1.0
75% of Maintenance Energy								
High Carbohydrate (2:1)	56	(10)	350	(60)	78	(30)	6.2	0.9
High Fat (1:1)	56	(10)	263	(45)	117	(45)	4.9	0.8

* The sample diet composition is given for a male of 66.1 kg body weight, with a maintenance energy requirement of 3100 kcal/d and 75% of maintenance energy equal to 2320 kcal/d.
† ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
‡ Determined for one menu day

validated by Bell et al. (1985). Protein intake was estimated at 0.82 g/kg/d protein — the recommended nutrient intake level (RNI) for a mixed Canadian diet (Dept. of National Health and Welfare, 1983). The absolute amount of protein (g/d) was kept constant among treatments. Thus the percentage of total energy supplied by protein differed slightly. Diets were formulated from food composition tables (Watt and Merrill, 1963; Dept. of National Health and Welfare, 1979) and the protein and energy content was confirmed by chemical analysis. Measured values for the individual diets were consistently higher for both protein (+15%) and energy (+5%) than the combined food values from the published tables. Thus, actual intakes are reported (Table 3.3) and used in all calculations requiring nutrient data. All meals were precisely weighed and

Table 3.3
Protein and energy intakes

Subjects	Protein*	Energy†	
	g/d ‡	*kcal/d*	*kcal/kg* §
KC	70	3490	50.2
PL	59	3060	50.2
JX	56	2870	52.6
HB	63	3130	50.2
NT	75	3465	45.4
JE	74	3510	47.8
X ± SEM	66 ± 3	3254 ± 111	49.4 ± 1.0

* Average intakes of four diet treatments.
† Average intakes of two diet treatments at maintenance energy.
‡ Values represent intakes of 1 g protein/kg body weight for each subject.
§ Values determined using initial body weights.

measured. A three-day rotating menu was followed to maintain strict control of the diet. The same foods were used for each diet period, only the quantity was altered for carbohydrate, fat and energy (Appendix I). Each diet was consumed for 14 days with a one-week break in between. The subjects tolerated the diets well.

Measurements and analyses

Venous blood samples were taken after a twelve-hour fast and one hour postprandial on days 1 and 14 of each diet period. The last six days of each diet represent a balance period when all urine and feces were collected for each 24-hour period, beginning at 8:00 a.m. daily. Homogenized aliquots of duplicate meals for each menu day were prepared and frozen. The diet samples and three-day pooled fecal samples were then freeze-dried to constant weight, ground and stored for analysis. Urine was preserved with 6 M HCl and frozen at -30°C.

Nitrogen content of diet, urine and fecal samples was analyzed by modified Kjeldahl method (Kjeltec System, Tecator, Hoganas, Sweden) to determine nitrogen balance. Urinary creatinine was measured as an indicator of completeness of urine collection (modified Jaffe method, Beckman Astra 8, automated Analyzer, Beckman Instruments, Fullerton, CA).

Blood levels of energy substrates and hormones were also determined. Measurements included glucose by hexokinase method (Schmidt, 1961) (Boehringer Mannheim Corp., Dorval, Canada), using an Abbott ABA 100 autoanalyzer, and triglycerides by enzymatic triglyceride method (Wahlefeld, 1974) (Boehringer Mannheim Corp., Dorval, Canada). Analysis of serum, using radioimmunoassay kits, was done for insulin (Pharmacia RIA Insulin 100, Pharmacia Diagnostics, Uppsala, Sweden), growth hormone (^{125}I-hGH

Chromacode double antibody method, Bio Mega Diagnostic Inc., Montreal, Canada), cortisol (Quanticoat RIA Kit, Kallestad Lab. Inc., Austin, TX), and triiodothyronine (Quanticoat T_3 Kit, Kallestad Lab. Inc., Austin, TX). Hematocrit was also measured (Coulter counter S+5).

The effects of energy level of the diet, dietary carbohydrate to fat composition and energy by diet composition interaction on nitrogen excretion and serum metabolite concentration were analyzed by repeated measures of analysis of variance procedures (Uanova Program, Computing Services, University of Alberta). The effect of diet quantity and type on the change in serum parameters from initial to final measurement and from fasting to fed state were analyzed by paired t-test (Steel and Torrie, 1980).

Results

Nitrogen balance

Nitrogen intake represented the mean of three menu days for each diet, based on total nitrogen content of the four meals, and it was constant in all treatments. Nitrogen excretion was the total of urinary and fecal nitrogen, plus an estimation of obligatory losses (sweat, skin, hair) (5 mg/kg body wt) (Calloway et al., 1971). Feeding the high carbohydrate diet increased total nitrogen excretion (per kg body weight) (Table 3.4) only at the maintenance energy intake level ($p < 0.05$).

Nitrogen balance was calculated (Table 3.5), and most subjects were in positive nitrogen balance for all four treatments. The greater nitrogen excretion observed when feeding the high carbohydrate/maintenance energy diet was attributed to a 12% increase in fecal and urinary losses. Figures 3.1 and 3.2 show the change in nitrogen balance between treatments for individual

subjects. Consistently higher daily nitrogen balance was observed for subjects fed the high fat diet (at maintenance energy only) ($p < 0.02$).

Table 3.4
Total daily nitrogen excretion

Cho:Fat Ratio	Nitrogen excretion*	
	g/d	*mg/kg/d*
Maintenance Energy		
High Carbohydrate (2:1)	10.56 ± 1.03†‡	158 ± 8.2†
High Fat (1:1)	9.38 ± 1.95	140 ± 6.3
75% of Maintenance Energy		
High Carbohydrate (2:1)	9.77 ± 1.21	149 ± 6.5
High Fat (1:1)	9.74 ± 0.79	149 ± 5.3

n=6 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
* Average of 6 days.
† Mean ± SEM.
‡ Different from high fat diet at maintenance energy ($p < 0.05$).

Table 3.5
Nitrogen balance of subjects on diet treatments

Cho:Fat Ratio	Nitrogen (mg/kg/d)				
	Intake	Output*†			Balance
	diet	*feces*	*urine*	*total*	
Maintenance Energy					
High Carbohydrate (2:1)	161 ± 4‡	27 ± 1	126 ± 9	158 ± 8	+ 3 ± 7§
High Fat (1:1)	158 ± 3	24 ± 1	111 ± 7	140 ± 6	+18 ± 8
75% of Maintenance Energy					
High Carbohydrate (2:1)	164 ± 5	24 ± 1	120 ± 7	149 ± 7	+15 ± 8
High Fat (1:1)	161 ± 3	22 ± 1	122 ± 6	149 ± 5	+12 ± 8

n=6 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
* Average of 6 days.
† Sweat losses = 5 mg N/kg body wt/d (23).
‡ Mean ± SEM.
§ Different from high fat diet at maintenance energy ($p < 0.02$).

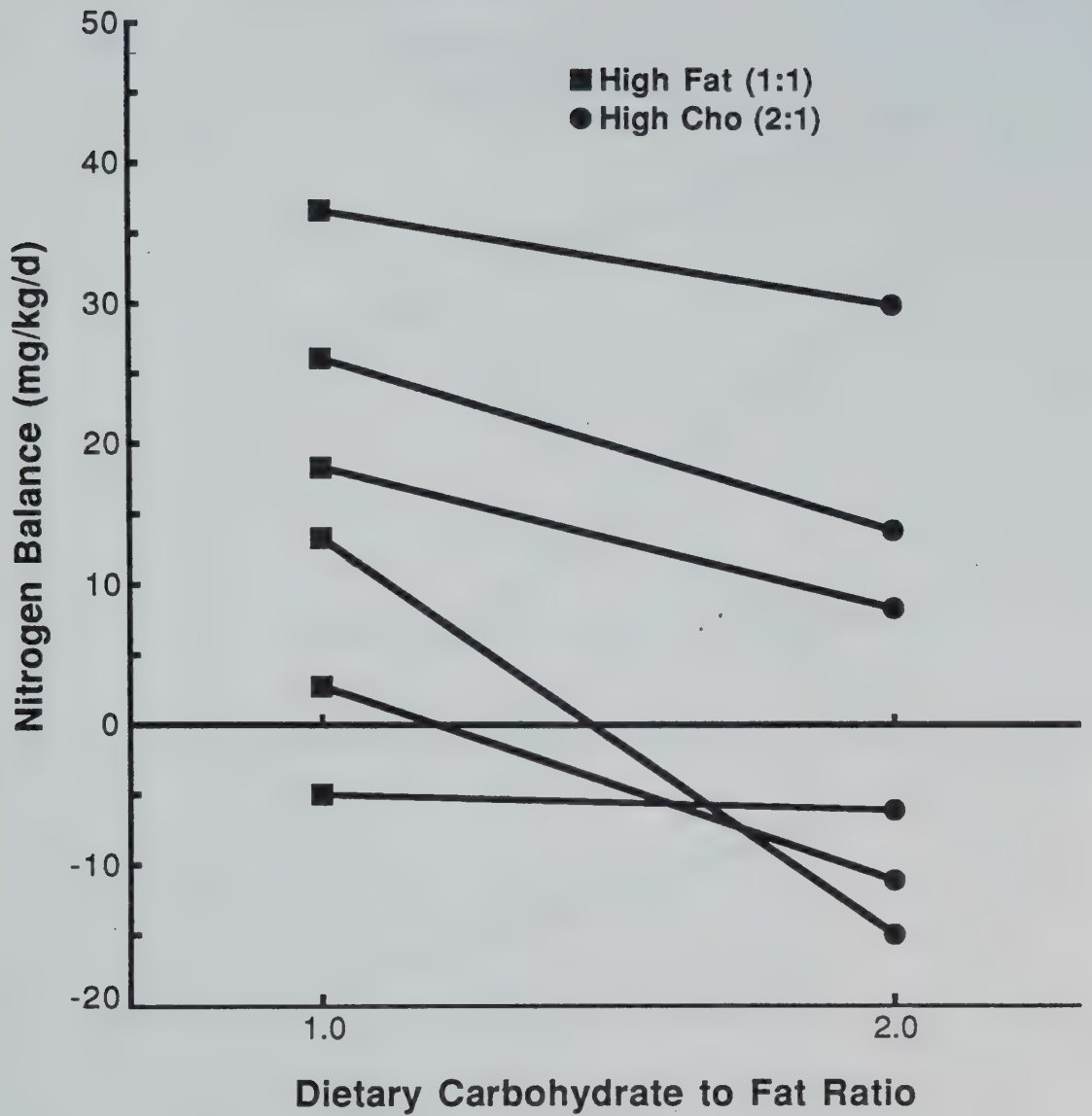

Figure 3.1 Nitrogen balance during two diet treatments at maintenance energy intake.

High Fat (1:1) = Dietary carbohydrate to fat ratio of 1:1.
High Cho (2:1) = Dietary carbohydrate to fat ratio of 2:1.

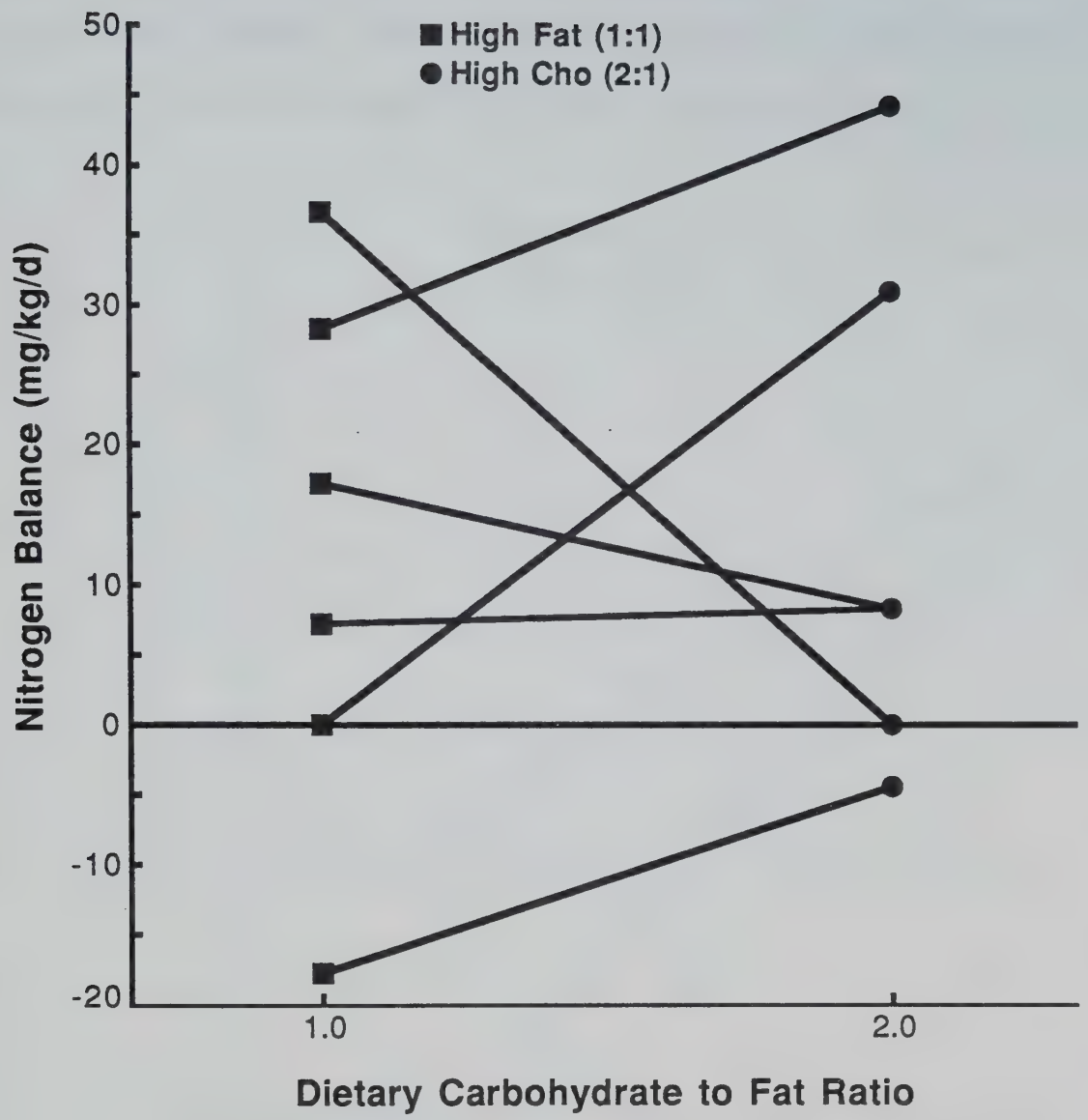

Figure 3.2 Nitrogen balance during two diet treatments at 75% of maintenance energy intake.

High Fat (1:1) = Dietary carbohydrate to fat ratio of 1:1.
High Cho (2:1) = Dietary carbohydrate to fat ratio of 2:1.

When nitrogen balance is plotted against energy intake at maintenance energy intake levels (Figure 3.3), the slope of the line shifts to the left for subjects consuming the high fat diet. However, the slopes were not significantly different either at maintenance or 75% maintenance energy intake (Figure 3.4). At maintenance energy, although zero nitrogen balance occurred at lower energy intake on the high fat diet, the rate of change (nitrogen balance per energy intake) was greater on the high carbohydrate diet.

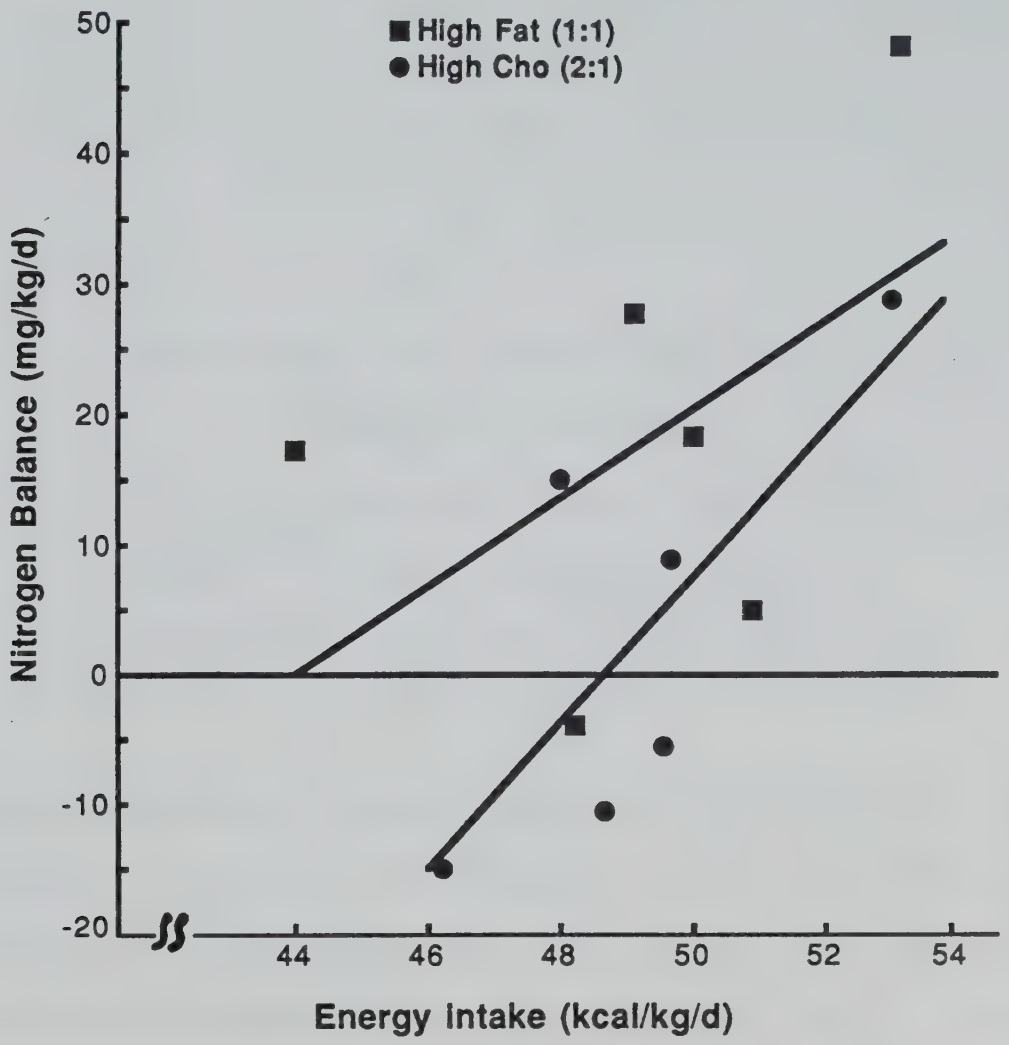

Figure 3.3 Nitrogen balance and energy intake at maintenance energy intake.
High Fat (1:1) = Dietary carbohydrate to fat ratio of 1:1
r = .55, y = -152.79 + 3.45 x
High Cho (2:1) = Dietary carbohydrate to fat ratio of 2:1
r = .77, y = -264.31 + 5.43 x

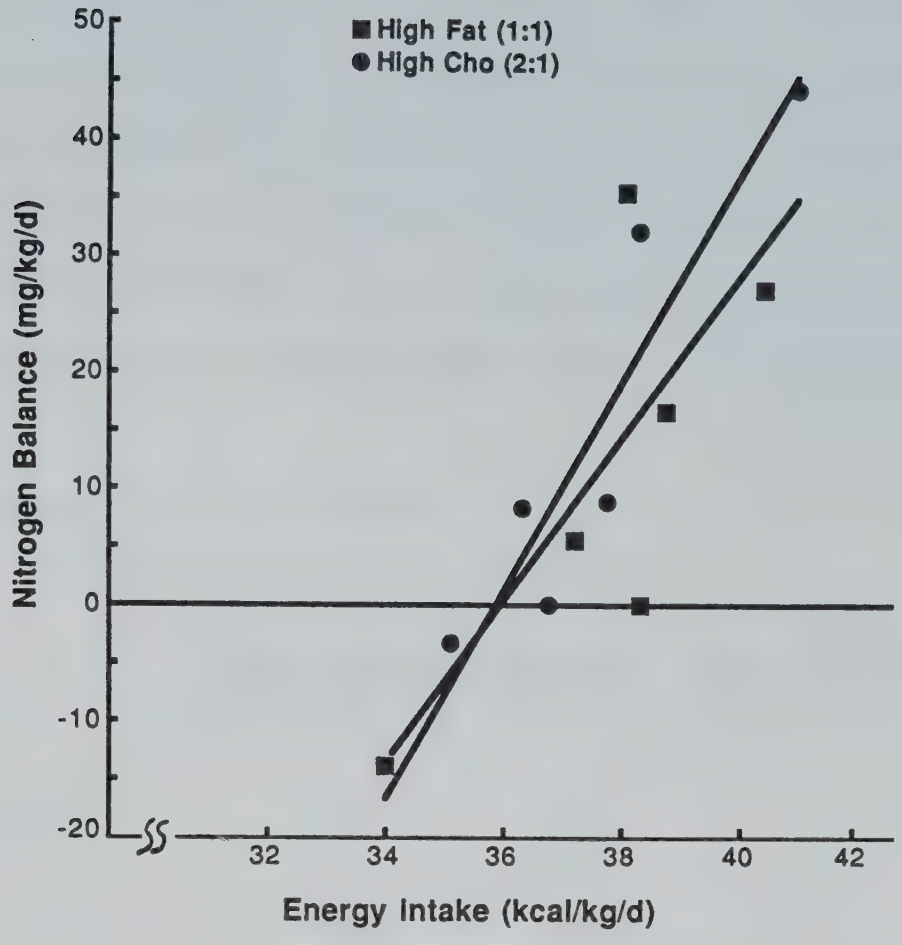

Figure 3.4 Nitrogen balance and energy intake at 75% maintenance energy intake.
High Fat (1:1) = Dietary carbohydrate to fat ratio of 1:1
r = .81, y = -233.50 + 6.51 x
High Cho (2:1) = Dietary carbohydrate to fat ratio of 2:1
r = .91, y = -295.11 + 8.24 x

Creatinine excretion remained stable over the entire study. The mean daily excretion for the six-day balance period was 12.0 ± 0.3 mmol and 12.4 ± 0.3 mmol for the high carbohydrate and high fat diets respectively (at maintenance energy intake), and 12.0 ± 0.5 mmol and 11.8 ± 0.4 mmol (at 75% of maintenance intake). Within each subject, the co-efficient of variation ranged from 2.7% to 13.5% between the four diet periods, consistent with variation reported for creatinine height indices (C.V. of 13.3%; Viteri et al., 1971).

Biochemical measurements

Blood parameters were measured on initiating each diet period to determine baseline values for each treatment. Where initial values on day 1 of each diet were different from final values on day 14, the change from initial to final was compared. Fasting (Table 3.6) and postprandial values (Table 3.7) measured at the end of each diet period are shown. Glucose values were not affected by diet treatment in the fasting or fed state. Fasting triglyceride levels

Table 3.6
Fasting serum blood parameters*

	Maintenance Energy		75% of Maintenance Energy	
	High Carbohydrate	High Fat	High Carbohydrate	High Fat
Glucose (mmol/l) (3.5-6.0)†	4.1‡ ± .1	4.4 ± .2	4.1 ± .1	4.1 ± .2
Triglycerides (mmol/l) (0.7-1.8)	1.61§ ± 1.10	1.20 ± .35	1.31 ± .54	1.13 ± .39
Insulin (mU/l) (5-20)	8 ± 2	8 ± 1	7 ± 1	8 ± .4
Triiodothyronine (nmol/l) (1.1-3.2)	2.6∞ ± .2	2.6 ± .2	2.3 ± .2	2.3⊥ ± .1
Growth hormone (µg/l) (< 5.0)	2.1 ± .1	2.1 ± .3	2.0 ± .1	2.1 ± .2
Cortisol (nmol/l) (100-700)	430 ± 145	524 ± 55	479 ± 54	502 ± 44

n=6
* Measured at the end of each diet period after a 12-hour fast.
† Normal values.
‡ Mean ± SEM.
§ Energy by diet composition interaction ($p < 0.01$).
∞ Difference by energy ($p < 0.05$).
⊥ Change from initial to final; high fat > high carbohydrate ($p < 0.05$).

Table 3.7
Postprandial serum blood parameters

	Maintenance Energy		75% of Maintenance Energy	
	High Carbohydrate	High Fat	High Carbohydrate	High Fat
Glucose (mmol/l) (3.5-9.0)†	4.0‡ ± .5	4.0 ± .4	4.3 ± .6	3.9 ± .3
Triglycerides (mmol/l) (0.7-1.8)	2.65 ± 3.33	2.09 ± .70	1.32 ± .65	1.45 ± .39
Insulin (mU/l) (5-20)	52 ± 24	28 ± 2	37§ ± 9	30 ± 5
Triiodothyronine (nmol/l) (1.1-3.2)	2.6 ± .3	2.6 ± .2	2.4 ± .3	2.4 ± .2
Growth hormone (µg/l) (< 5.0)	2.0 ± .1	2.1 ± .4	1.9 ± .1	1.8 ± .1
Cortisol (nmol/l) (100-700)	389 ± 95	386 ± 28	341 ± 14	394 ± 40

n=6
* Measured at the end of each diet period after a 12-hour fast.
† Normal values.
‡ Mean ± SEM.
§ Change in fasting to fed; high carbohydrate > high fat ($p < 0.05$).

showed asignificant diet composition by energy level interaction ($p < 0.01$). Triglyceride values were higher for subjects fed the high carbohydrate diet and at high energy intake. A similar, but insignificant effect of energy intake level was seen in the fed state. No effect of diet composition was seen.

One subject had very high triglyceride values throughout all diet treatments, ranging from 2.37 to 9.39 mmol/l (normal = 0.7 to 1.8 mmol/l). With exclusion of his results, the overall same elevation in fasting triglycerides for subjects fed the high carbohydrate diet was observed. Without this subject,

postprandial triglyceride values were normal (1.30 ± 2.2 versus 1.41 ± .19 mmol/l for subjects fed high carbohydrate and high fat diets respectively, at high energy intake levels; and 0.68 ± .11 versus 1.07 ± .10 mmol/l for subjects fed high carbohydrate and high fat diets, respectively, at low energy intake levels).

Fasting insulin values showed no difference among treatments. At both energy intakes, the change from fasting to fed state was higher for subjects fed the high carbohydrate diet and was statistically significant at 75% energy intake level ($p < 0.05$). High carbohydrate meals stimulated a greater insulin release, particularly at the end of the diet period.

Fasting triiodothyronine (T_3) values differed, with higher values observed at high energy intakes ($p < 0.05$). A similar trend was demonstrated in the fed state. The change in fasting serum T_3 from initial to final level was greater for subjects fed the high fat diet, but only at 75% of maintenance energy intake ($p < 0.05$).

Growth hormone and cortisol showed no significant change in either the fasting or fed states, in response to diet treatments. The high fat diet resulted in a consistent trend towards higher fasting cortisol levels at high and low energy intakes, and also higher postprandial cortisol levels at low energy intake levels.

Discussion

It has been suggested that the response of protein turnover to nutrient intake is influenced by two possible mechanisms: an indirect hormonal effect, or a more direct substrate effect. Difficulties in interpretation occur because of the interaction of various hormones, and it is not known if small changes (still

within physiological levels of the hormone) can affect protein metabolism significantly.

Blood concentrations of glucose were unchanged by diet composition or by energy level. No change in blood glucose concentration was seen in pigs fed high carbohydrate diets (Reeds and Fuller, 1983), or in healthy male subjects (Richardson et al, 1979). Glucose is a tightly regulated substrate and blood levels are often not a good indicator of its flux. Presumably, serum glucose levels were higher on the high carbohydrate diets, but the subsequent increased insulin release would have cleared the substrate proportionately. The excess glucose would be converted to fat.

At both energy levels, fasting triglyceride concentrations were lower with the high fat diet. This was also shown by Richardson et al. (1979). Measurement of fasting triglycerides is recommended because of difficulty in interpreting a single casual measurement (Simons and Gibson, 1980) which may be greatly influenced by food, exercise, or stress. The lower serum triglyceride levels on the high fat diets could be due to greater fat storage (Sauer et al., 1986), increased fat oxidation or the triglyceride lowering effect of increased polyunsaturated fats (Grundy, 1975). The high fat diets were mainly supplemented with a high polyunsaturated margarine. The elevated triglycerides on the high carbohydrate diet may be a reflection of increased endogenous synthesis. Carbohydrate-induced hypertriglyceridemia may occur if, after energy and glycogen storage demands have been met, there is excess glucose for hepatic lipogenesis (Simons and Gibson, 1980).

Various hormones have been suggested as the stimulus for changes in nitrogen metabolism due to changes in diet. Originally, Munro (1964) stated that high dietary carbohydrate resulted in increased insulin secretion, which led to increased amino acid uptake and increased protein synthesis. A high

carbohydrate diet has greater influence on insulin levels and on lipogenesis (Heymsfield et al., 1984). Woolfson et al. (1979) showed increased nitrogen retention with intravenous glucose and insulin compared to glucose alone, or lipid and sorbitol. The greater differences were found with increased severity of illness, believed to be due to the glucose and/or insulin resistance in such patients. Insulin deficiency also results in increased protein degradation (Neely and Morgan, 1981; Albertse, 1980).

Insulin levels were higher after feeding the high carbohydrate diets. The amount of insulin necessary for maximum protein utilization, however, is small. Jeejeebhoy (1981) has suggested that it is as little as that available in the fasting state. Fasting insulin did not vary among treatments.

Energy level had a significant effect on fasting T_3 levels. Values were higher on the high energy diets, regardless of composition. It has been documented that high carbohydrate intakes increase serum concentrations of T_3 in man (Davidson and Chopra, 1979; Danforth et al, 1979). It is suggested that the increased production of T_3 stimulates thermogenesis (Danforth et al, 1979), and thus the increased energy expenditure seen with high carbohydrate diets is, in part at least, modulated by the thyroid hormone. Hyperthyroidism is associated with decreased skeletal mass (Buttery, 1983). However, decreased availability of T_3 reduces muscle protein synthesis (Brown et al., 1981), which returns to normal with restoration of thyroid status (Goldberg et al., 1980). With further increases in thyroid hormones, more extensive muscle catabolism occurs (Goldberg et al., 1980). It is suggested that at physiological concentrations, thyroid hormones play an important role in muscle protein deposition. In this study, T_3 values did not differ due to diet composition except at 75% maintenance, where the the increase from initial to

final measurement was significantly higher on the high fat diet. This did not appear to influence nitrogen retention.

Growth hormone and cortisol showed no change with the diet treatments, either fasting or postprandial. Growth hormone has been shown to increase muscle protein and improve nitrogen retention (Eisenstein and Singh. 1980). Corticosteroids stimulate mobilization of protein stores. When secreted in response to injury, a decrease in protein synthesis rate occurs with no change in protein breakdown rate (Millward, 1976). Cortisol values were generally lower on the high carbohydrate treatments, because carbohydrate inhibits cortisol release. Perhaps internal shifts in protein metabolism occurred with the higher cortisol levels, but net nitrogen excretion may not be a sensitive enough measurement to represent subtle hormonal changes. It may be that attention should be given to the balance between anabolic and catabolic hormones (Reeds and Fuller, 1983) and their concentrations at different time-points after a meal. Examination of the changes in protein synthesis and degradation may also be necessary to find a more definitive description of the diet, hormone and protein turnover relationship.

Negative nitrogen balance occurs if protein or energy intakes are too low or if there is increased protein catabolism. Nitrogen balance appears to occur at energy intakes of about 40 kcal/kg body weight in normal subjects (Munro, 1964; Wolfe et al., 1977). Richardson et al. (1979) found that subjects required higher energy intakes (43 to 48 kcal/kg) to achieve nitrogen balance, but in their study protein intake was quite low (0.56 g/kg body wt). This was also observed in the present study, where maintenance energy intakes of 44 to 48 kcal/kg were required.

All subjects had adequate protein intakes, yet even at maintenance energy some subjects showed a negative nitrogen balance on the high

carbohydrate diet (those whose energy intake was less than the group mean). On the high fat diet, one subject was in negative nitrogen balance (energy intake = 48 kcal/kg). On the low energy diets, where intakes averaged 38 ± 2 kcal/kg, weight loss occurred but nitrogen balance was not affected. Only one subject showed a negative nitrogen balance of -4 mg/kg/d on the high carbohydrate diet, and -14 mg/kg/d on the high fat diet. His energy intakes were below the group mean at 35 and 34 kcal/kg respectively. On the low energy diets, adaptive mechanisms for conservation of body protein may have been triggered and overridden an effect of diet composition. The high nitrogen balance values observed during the low energy intakes may also reflect a change in the energy to protein ratio. At 75% maintenance, protein provided 10% of total energy, compared to 7% at maintenance energy. Thus, the proportion of protein and energy may be more critical than the absolute energy level in terms of nitrogen balance.

The regression equations of nitrogen balance and energy intake showed that the magnitude of the increase in nitrogen balance per increase in kcal energy intake was greater when subjects were fed the high carbohydrate diet at both energy levels. It is not known if the mean change or the rate of change is more important over the long term.

Studies of the the effect of the non-protein energy source on protein metabolism have led to inconsistent results. In the present studies at maintenance energy intake, when positive nitrogen and energy balance were achieved (except one subject) and the diet non-protein energy source was the only variable manipulated, the high fat diet led to protein-sparing (i.e., the reduction in excretion of nitrogen compounds in the urine) (Wolfe et al., 1977). In growing rats fed isocaloric diets at different fat to carbohydrate ratios, fecal nitrogen and urinary nitrogen excretion was less with the high fat diet compared

to the high carbohydrate diet at two levels of protein intake (Hartsook et al., 1973). Richardson et al. (1979) found higher nitrogen balance values with a high fat diet (carbohydrate:fat at 1:1) when daily energy intake was greater than 50 kcal/kg body weight, but not at lower intakes. In intravenous feeding studies, there have been reports that favor a high fat energy source (Pineault et al., 1985; Macfie et al., 1981), a high carbohydrate energy source (Long et al., 1977; Nordenstrom et al., 1983), and some find both substrates of equal value for their effects on protein status.

Nordenstrom et al. (1983) have suggested that carbohydrate influences protein sparing only at low intakes (i.e., glucose 100 to 150 g/d), and that after obligatory requirements for carbohydrate are met (2 to 4 g/kg/d), then fat and carbohydrate energy sources have equal effect on nitrogen balance. This does not explain the differences in the present study, because obligatory glucose requirements were met on all diets.

Creatinine is an indirect measure of nitrogen metabolism. If renal function is normal and fluid intake is sufficient, free creatinine is released from muscle at a relatively constant rate in proportion to muscle mass (Kaminski and Jeejeebhoy, 1979). When chronic muscle wasting occurs, the skeletal muscle mass and creatinine excretion both decrease (Forbes and Browning, 1976; Blackburn et al., 1979). There was no difference in creatinine excretion between diets or between energy levels. It appears that a longer time period, or a more extreme dietary treatment, is necessary to see a decreased creatinine excretion.

There is evidence to show that very small changes in protein turnover have the potential to produce marked changes in nitrogen retention (Reeds and Fuller, 1983), and that separate diet components affect mechanisms that link nutrient intake to protein synthesis and degradation in different ways. The

question may be how a high fat diet causes less net nitrogen loss, or how a high carbohydrate diet increases nitrogen losses. It has been shown that a high carbohydrate diet increases O_2 consumption and CO_2 production (Sauer et al., 1986; Acheson et al., 1984). Consequently, resting energy expenditure also increases. About 15% of resting energy expenditure comes from the oxidation of amino acids (Woolfson, 1983). If this is a true proportional effect based on resting energy expenditure, then the absolute amount of amino acid oxidation and nitrogen excretion would increase on a high carbohydrate diet. Also, amino acid oxidation may be spared on the high fat diet, as more fatty acids are used for energy. Considering the close relationship between protein and energy metabolism, and the fact that protein and energy intakes were the same, it may be that the difference between the diets is due to the effect on energy expenditure.

Some studies have shown that high fat diets are energetically more efficient than high carbohydrate diets, that heat production decreases and fat deposition increases. It is suggested that increased nitrogen retention may represent the protein required to support the increased fat depots.

When calculated nitrogen gains or losses are small, these may persist for long periods with minimal implications in terms of body weight or function (Hegsted, 1976). Examination of this question may require longer diet treatment periods to see consistent effects on biochemical parameters. It also appears that the components of energy balance, both intake (based on kcal/kg body wt) and expenditure (changes in O_2 consumption due to diet composition), play an important role in man's nitrogen economy and should be addressed further (Chapter 4).

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Chapter 4.
Influence of Dietary Carbohydrate to Fat Ratio on Body Composition, Substrate Utilization and Energy Expenditure in Healthy Male Subjects

Introduction

In studies on parenteral and enteral feeding, it has been found that altering the non-protein energy source can affect substrate utilization and energy expenditure (Heymsfield et al., 1984; Sauer et al., 1984, 1986; Nose et al., 1985). Consequently, over the long term, body composition may also be influenced. In infants, fat deposition increased when a lipid source was provided in the diet, compared to a glucose and amino acid solution, even when total energy was kept constant (Sauer et al., 1986).

The metabolic changes observed with parenteral feedings may also occur with oral feedings. If dietary fat is utilized more efficiently such that energy expenditure is decreased, this could have implications for weight control (Bray, 1984; Lissner et al., 1987). Certainly, the high fat diets of Western societies appear to be stored very efficiently as indicated by the high prevalence of obesity (Millar and Stephens, 1987) in countries such as Canada and the United States where fat intake averages 40-42% of total energy (Dept. of National Health and Welfare, 1977; Select Committee on Nutrition and Human Needs, 1977). Not only the quantity but also the quality of the macronutrients may play an important role in energy balance.

The purpose of the present study was to evaluate substrate utilization, energy expenditure and body composition of healthy male subjects fed a high carbohydrate (Cho:Fat 2:1) and a high fat (Cho:Fat 1:1) diet. Both diets were fed at a level estimated to provide maintenance and 75% maintenance energy requirements.

* A combined version of Chapters 3 and 4 has been accepted for publication:

McCargar L.J., Clandinin, M.T., Belcastro, A.N. and Walker, K. Influence of dietary carbohydrate to fat ratio on whole body nitrogen retention, substrate utilization and hormone response in healthy male subjects (accepted, Am. J. Clin Nutr. 1988).

Materials and Methods

Subjects and experimental design

The subjects were six young male university students. Criteria for participation in the study required sedentary, non-smoking subjects, free from chronic illness, having no requirement for medication, and weighing less than 77.0 kg with a body fat content between 10 and 20%. Initial assessment included a medical history, lifestyle questionnaire, diet and activity history along with anthropometric measurements. The purpose and requirements of the study were fully explained to each subject before they gave informed consent. The study was approved by the Ethics Review Committee for Human Experimentation, Faculty of Medicine, University of Alberta.

During the study, subjects followed their usual routine, except they reported daily to the metabolic laboratory to be weighed and to eat breakfast (8:00 a.m.) and lunch (12:00 p.m.). A pre-packaged supper and snack were provided to be taken with them. The four daily meals were isocaloric and isonitrogenous.

Each subject received four different diet treatments. The non-protein energy was supplied at two ratios of carbohydrate and fat (2:1 and 1:1) randomly assigned, and at two energy levels (maintenance and 75% maintenance requirements) (Table 3.2). Energy requirement was determined using the Mayo Clinic Nomogram (Food Nomogram, 1981) by a method validated by Bell et al. (1985). Protein intake was estimated at 0.82 g/kg/d protein — the recommended nutrient intake level (RNI) for a mixed Canadian diet (Dept. of National Health and Welfare, 1983). Diets were formulated from food composition tables (Watt and Merrill, 1963; Dept. of National Health and Welfare, 1979) and the protein and energy content was confirmed by chemical analysis. All meals were precisely weighed and measured. A three-day

rotating menu was followed to maintain strict control of the diet. Each diet was consumed for 14 days with a one-week break in between. The subjects tolerated the diets well.

Measurements and analyses

During the last six days of each diet (balance period), all urine and feces were collected for each 24-hour period beginning at 8:00 a.m. daily. Homogenized aliquots of duplicate meals for each menu day were prepared and frozen. The diet samples and three-day pooled fecal samples were then freeze-dried to constant weight, ground and stored for analysis. Urine was preserved with 6M HCl and frozen at -30°C.

Energy content of diet and fecal samples was determined by bomb calorimetry (Model #1241, Parr Instrument Co., Moline IL). Anthropometric measurements comprising height, weight, mid upper arm circumference and skinfold thickness at four sites (biceps, triceps, subscapula, suprailiac) were done initially and at the end of each diet period. Lange caliphers (Cambridge Scientific Industries, Cambridge, MD) were used for skinfold thickness measurements, and each measurement was repeated three times on each occasion by a single trained observer. Measurements with this technique are accurate within ± 5% (Durnin and Rahaman, 1967). Percentage body fat was then calculated from the skinfold thickness values (Durnin and Womersley, 1974).

Oxygen consumption measurements were done at rest and for 2 1/2 hours postprandial at the beginning and end of each diet period. The initial measurements were to familiarize the subjects with the procedure and to obtain baseline values for each diet period. Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory quotients (RQ) were determined

with the subject sitting comfortably, breathing into a mouthpiece (one-way valve) and hose which was attached to a metabolic measurement chart (Beckman Instruments, Fullerton, CA).

Oxygen content of air was analyzed by a Beckman OM-11 oxygen analyzer and CO_2 content by a Beckman LB-2 medical gas analyzer. Automatic programs were available for immediate calculations of VO_2, VCO_2 and RQ (Monroe Model 1810 programmable printing calculator).

Three sets of measurements (five readings per set) were taken 15 minutes apart in the fasted state to obtain an average resting energy expenditure. Resting energy expenditure has been defined as the energy expended at a time when a subject is physically inactive during the day, a minimum of three hours after eating, but after metabolism has been stimulated. It generally is about 20% greater than basal metabolism (Flatt, 1977). The caloric value for resting energy expenditure was calculated from the formula by Weir (1949), using measured values of VO_2, VCO_2 and grams of urinary nitrogen. Nine sets of measurements (five readings per set) were taken 15 minutes apart in the fed state after a standard test breakfast. Values for VO_2 are presented at half-hour time points to represent the thermic effect of feeding. The thermic effect of feeding is the transient increase in metabolic rate which occurs after a meal (Garrow, 1978; Welle et al., 1980). It represents the energy cost of digestion, absorption, transport, and storage of the nutrients of the meal and results in an estimated 10% increase in heat production on a mixed diet (Flatt, 1977; Himms-Hagen, 1981).

Urinary nitrogen values were used to determine the non-protein RQ (Frayn, 1983). From this value, the oxidation of carbohydrate and fat were calculated according to the tables of Lusk (1924).

The effects of energy level of the diet, dietary carbohydrate to fat composition, and energy by diet composition interaction on body composition and energy utilization parameters were analyzed by repeated measures of analysis of variance procedures (Uanova Program, Computing Services, University of Alberta). The effect of diet quantity and type on the change in parameters from initial to final measurement were analyzed by paired t-test (Steel and Torrie, 1980).

Results

Bomb calorimetry

Actual energy content of the diets was higher (+5%) than that predicted from published table values, but there was no difference between either diet treatment at each energy intake level (Table 4.1). Fecal energy losses were higher at maintenance energy intakes than at 75% maintenance intakes ($p < 0.05$). No difference in fecal energy loss occurred in response to changing the carbohydrate to fat ratio in the diet at maintenance energy (Table 4.1). Thus, digested energy (diet energy minus fecal energy) was similar between treatment groups. At 75% maintenance intakes, fecal losses were slightly higher with the high fat diet ($p < 0.05$), resulting in an energy by diet composition interaction.

Anthropometric measurements

At maintenance energy intake levels, feeding the high fat diet resulted in a slightly increased weight gain (Δ + 0.4 kg) compared to the high carbohydrate diet (Δ + 0.2 kg) (Table 4.2). At 75% maintenance energy intake, weight loss occurred as expected, but it was significantly greater ($p < 0.05$) on the high carbohydrate diet (Δ – 1.4 kg) compared to the high fat diet

Table 4.1
Energy value of dietary intake and fecal losses during diet treatment

Subject	Maintenance Energy		75% of Maintenance Energy	
	High Carbohydrate	High Fat	High Carbohydrate	High Fat
	kcal/d			
Diet Energy *				
KC	3561	3442	2677	2581
PL	3035	3083	2271	2318
JX	2844	2868	2127	2151
HB	3083	3155	2295	2366
NT	3537	3394	2653	2557
JE	3466	3561	2581	2677
X ± SEM	3254 ± 124	3251 ± 106	2434 ± 95	2442 ± 80
Fecal Energy †‡				
KC	186	186	213	201
PL	189	170	146	203
JX	158	148	139	129
HB	203	218	191	198
NT	201	230	184	201
JE	186	171	141	143
X ± SEM	187 ± 7	187 ± 13	169 ± 13	179 ± 8

n=6
* Difference by energy (high energy > low energy) ($p < 0.001$).
† Difference by energy (high energy > low energy) ($p < 0.05$).
‡ Energy by diet composition interaction ($p < 0.05$).

Table 4.2
Anthropometric measurements after diet treatment

Cho:Fat Ratio	Body weight initial	Body weight final	Weight change	Body mass index	Body fat
	kg			*BMI* *†	*%**
Maintenance Energy					
High Carbohydrate (2:1)	66.2 ± 3.5‡	66.4 ± 3.5§	+ 0.2	21.4 ± .7∞	16.7 ± 1.6
High Fat (1:1)	66.1 ± 3.6	66.5 ± 3.3§	+ 0.4	21.4 ± .7∞	16.7 ± 1.5
75% of Maintenance Energy					
High Carbohydrate (2:1)	66.1 ± 3.5	64.7 ± 3.3	− 1.4⊥	20.9 ± .7	16.5 ± 1.7
High Fat (1:1)	65.6 ± 3.2	64.9 ± 3.2	− 0.7	20.9 ± .6	16.4 ± 1.6

n=6 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
* Measured at the end of each diet period.
† BMI = wt (kg)/ht (m^2).
‡ Mean ± SEM.
§ Difference by energy (high energy > low energy) ($p < 0.02$).
∞ Difference by energy (high energy > low energy) ($p < 0.01$).
⊥ Difference from high fat diet (@75% maintenance energy) ($p < 0.05$).

(Δ − 0.7 kg). Body mass index showed no effect of diet composition, but a decrease was observed with decreased energy intakes ($p < 0.01$). Percentage body fat showed a slight decrease with decreased energy.

Indirect calorimetry

Resting energy expenditure was lower during the high fat diet period by an average of 114 kcal/d at maintenance energy intake levels and 120 kcal/d at 75% maintenance energy intake levels (Table 4.3). The same trend occurred when expressed per kilogram body weight, but these differences did

Table 4.3
Resting energy expenditure during diet treatment*

Cho:Fat Ratio	Resting energy expenditure REE†		Respiratory quotient (RQ)
	kcal/d	*kcalkg*	*(VCO_2/VO_2)*
Maintenance Energy			
High Carbohydrate (2:1)	1563 ± 107‡	24.0 ± 0.8	.84 ± .05§
High Fat (1:1)	1451 ± 229	21.7 ± 2.5	.78 ± .04
75% of Maintenance Energy			
High Carbohydrate (2:1)	1525 ± 181	23.4 ± 1.7	.79 ± .05
High Fat (1.1)	1403 ± 182	21.5 ± 1.8	.75 ± .03

n=5 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
* Measured at the end of each diet period.
† Calculated by the formula of Weir (1949).
‡ Mean ± SEM
§ Different from high fat diet (maintenance energy) ($p < 0.05$).

not reach statistical significance. The mean resting RQ values were lower for subjects fed the high fat diets, with a significant difference at high energy intakes ($p < 0.05$).

Mean oxygen consumption values were slightly higher on the high carbohydrate diets compared to the high fat diets, with the exception of the 1 h and 1 1/2 h postprandial time points at maintenance energy and 1/2 h for 75% maintenance energy intake levels (Table 4.4). The test breakfast provided the same macronutrient composition as other isocaloric meals of the specific diet being tested. The thermic effect of feeding, in response to test meals at

Table 4.4
Changes in postprandial oxygen consumption after diet treatment

Cho:Fat Ratio	Oxygen consumption (VO_2 – ml/min)					
	rest*	1/2 h†	1 h	1 1/2 h	2 h	2 1/2 h
Maintenance Energy						
High carbohydrate (2:1)	226 ± 17	256 ± 34	249 ± 32	254 ± 33	258 ± 34	267 ± 21
High fat (1:1)	212 ± 33	239 ± 24	253 ± 37	261 ± 41	250 ± 39	252 ± 35
75% of Maintenance Energy						
High carbohydrate (2:1)	224 ‡ ± 28	251 ± 21	262 ± 28	254 ± 30	252 ± 25	240 ± 17
High fat (1:1)	207 ± 26	251 ± 25	257 ± 27	249 ± 33	244 ± 21	228 ± 21

n=5 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
Mean ± SEM
* Fasting (12h).
† 1/2 hour after eating.
‡ Different from high fat diet (@ 75% maintenance energy) ($p < 0.05$).

maintenance energy, was still increasing at 2 1/2 h, whereas after feeding test meals at 75% maintenance energy level, the oxygen consumption was returning to resting values by 2 1/2 h (Table 4.4). Mean carbon dioxide production values were not significantly different but tended to be higher on the high carbohydrate diets at all time points for both energy levels (Appendix II).

Mean postprandial respiratory quotients showed a trend toward higher values for subjects fed high carbohydrate diets (Figures 4.1, 4.2). The

difference was significant ($p < 0.05$) at all time points, except 1/2 h for maintenance energy intake levels, and at rest, 1 h and 2 1/2 h for 75% maintenance energy intake levels. A larger amount of fat and less carbohydrate was apparently oxidized for subjects fed the high fat diets. The non-protein respiratory quotients were higher with high carbohydrate feeding (Table 4.5) when subjects were fed maintenance energy diets (resting values $p < 0.05$) and 75% maintenance diets (2h postprandial values $p < 0.07$).

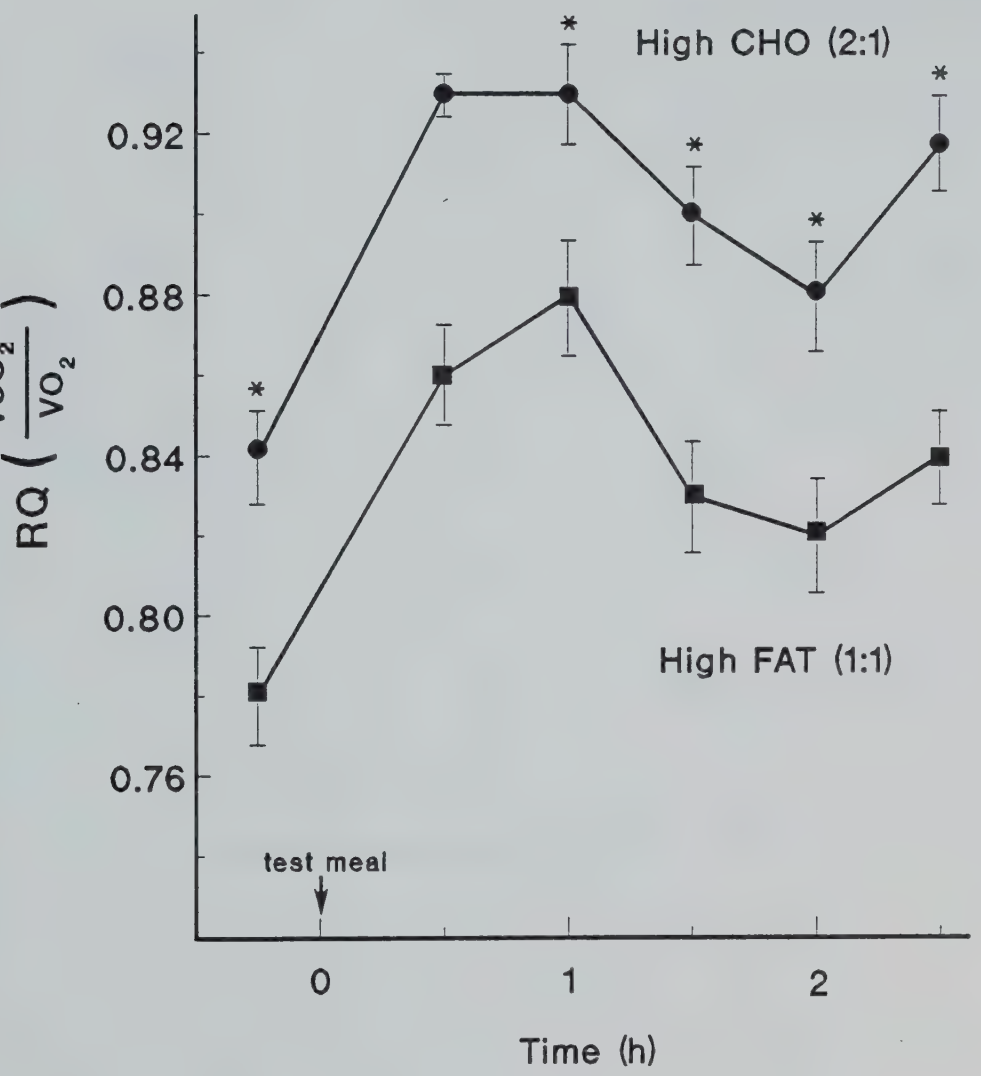

Figure 4.1 Fasting and postprandial RQ at maintenance energy intake.
High Fat (1:1) = Dietary carbohydrate to fat ratio of 1:1.
High Cho (2:1) = Dietary carbohydrate to fat ratio of 2:1.
* $p < 0.05$; paired t-test.
Protein oxidation is not subtracted from RQ (i.e., not non-protein RQ).

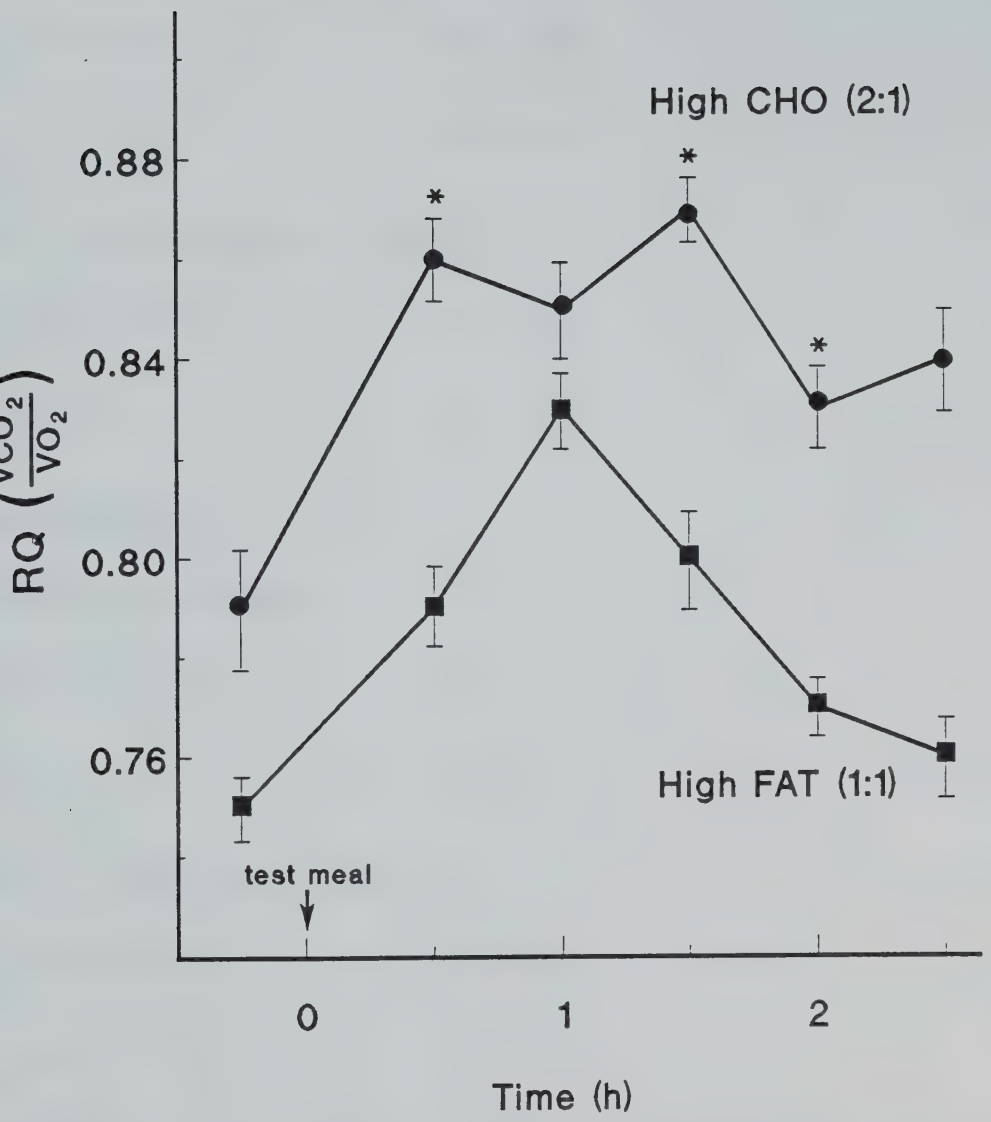

Figure 4.2 Fasting and postprandial RQ at 75% of maintenance energy intake.
High Fat (1:1) = Dietary carbohydrate to fat ratio of 1:1.
High Cho (2:1) = Dietary carbohydrate to fat ratio of 2:1.
* $p < 0.05$; paired t-test.
Protein oxidation is not subtracted from RQ (i.e., not non-protein RQ).

Table 4.5
Non-protein respiratory quotients (NPRQ) and percentage of oxygen consumed by carbohydrate and fat*

Cho:Fat Ratio	NPRQ	% of total O_2 consumed by carbohydrate†	fat†
At rest			
Maintenance Energy			
High Carbohydrate (2:1)	.85 ± .06‡	48.8	51.2
High Fat (1:1)	.76 ± .05	18.1	81.9
75% of Maintenance Energy			
High Carbohydrate (2:1)	.78 ± .05	24.9	75.1
High Fat (1:1)	.73 ± .03	7.8	92.2
2h postprandial			
Maintenance Energy			
High Carbohydrate (2:1)	.89 ± .06	62.5	37.5
High Fat (1:1)	.82 ± .07	38.6	61.4
75% of Maintenance Energy			
High Carbohydrate (2:1)	.83 ± .04‡	42.0	58.0
High Fat (1:1)	.76 ± .05	18.1	81.9

n=5 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
* Measured at the end of each diet period.
† Calculated by the tables of Lusk (1924). Mean values used for calculations, thus no standard errors.
‡ Different from high fat diet ($p < 0.07$).

Discussion

Energy intake influences many components of energy expenditure. An increase or decrease in the amount of energy in the diet will respectively increase or decrease energy expenditure under normal conditions (Garrow, 1978; Garlick et al., 1980). It also appears that the source of energy in the diet may play a major role in energy expenditure. There is evidence to suggest that high fat diets lead to a decrease in energy expenditure, an increase in fat oxidation (decrease in RQ), and over the long term an increase in fat deposition, compared to high carbohydrate diets of the same caloric value.

In parenterally fed infants (Sauer et al., 1986; Nose et al., 1985) and enterally fed adults (Heymsfield et al., 1984), when lipid was substituted for a portion of the glucose calories, energy expenditure decreased, fat oxidation increased and fat deposition increased (Sauer et al., 1986), compared to when only carbohydrate and amino acids were fed.

Basal metabolism was lower in rats fed high fat diets (Fat:Cho .8-1.4) compared to low fat diets (Fat:Cho .2-.5), but only at high levels of protein intake (> 38.5% of energy) (Hartsook et al., 1973). Rats fed high fat compared to low fat diets exhibited less heat production (at protein intakes of 7-22%) (Black et al., 1949). In the present study, the trend toward lower resting energy expenditure and postprandial O_2 consumption and CO_2 production measurements on the high fat diet support the same effect.

Energy expenditure is approximately proportional to oxygen consumption. Kleiber (1975) demonstrated a relatively constant relationship between heat release and O_2 consumed. Energy expenditure can be calculated based on respiratory exchange and urinary nitrogen excretion, and because substrate oxidation and ATP production are tightly coupled,

determination of energy expenditure also represents a measure of ATP production (Flatt, 1977).

Energy expenditure is mainly determined by the cost of resting metabolism (Garrow, 1978). Two factors involved in its control include the costs of nutrient conversion for energy storage and changes in body weight and body composition (Garrow, 1978). Conversion of dietary fat to triglycerides for use by body tissues is energetically a very efficient process (Heim, 1981). It has been estimated that only 2 - 7% of the energy of the substrate is required for synthesis of the end product (Flatt, 1977; Heim, 1981; Leveille and Cloutier, 1987). The cost of conversion of dietary carbohydrate to fat has been estimated at 24% (Flatt, 1977). On a high fat diet, any excess fat would be stored very efficiently. The excess glucose on a high carbohydrate diet would be stored inefficiently through lipogenesis after glycogen demands are met. One reason for increased energy expenditure on a high carbohydrate diet may be increased lipogenesis. Fasting non-protein RQ shows a strong positive relationship with net lipogenesis ($r = .82$), and lipogenesis results in a strong positive relationship with energy expenditure ($r = .71$) after a carbohydrate load (Acheson et al., 1984).

Another component of energy expenditure affected by nutrient supply is the thermic effect of feeding. Although estimates vary, it is generally agreed that carbohydrate elicits a 5-10% increase in metabolism compared to fat at a 0-3% increase (Flatt, 1977). Over a longer time period, the cumulative effect of the thermic effect of feeding between high carbohydrate and high fat diets may be substantial. These subjects had slightly higher response (3 - 4%) with the higher carbohydrate intakes at both energy levels. Postprandial oxygen consumption values continued to increase after 2 1/2 hours on the high energy diets and a longer measurement period is indicated. Welle has stated that

thermic responses are only clearly differentiated 2-3 hours after ingestion, and that measurement of metabolic rate for greater than 4 hours is required for complete quantification of the thermic effect of fat (Welle et al., 1981). Ideally, 24 hour measurement of oxygen consumption is preferable to determine diet influence on total daily energy expenditure.

Ingestion of glucose (Welle et al., 1980) or fat (Schwartz et al., 1983) has been shown to stimulate norepinephrine turnover, which increases metabolic rate (Welle et al., 1980). Glucose and fat intake resulted in an equal thermic effect with a response lower than the effect of protein (Welle et al., 1981). This differs from the report by Flatt (1977). It appears unlikely that the difference in energy output between the two diet compositions is mediated by sympathetic activity.

Carbohydrate is also known to stimulate thyroid hormone production (Davidson and Chopra, 1979; Danforth et al., 1979), which subsequently stimulates thermogenesis (Danforth et al., 1979). Thus, the increased energy expenditure observed with high carbohydrate diets may be due to increased thyroid hormone release. In this study, however, T_3 values showed no difference due to diet composition at the time-points measured (Chapter 3).

At rest, the proportions of carbohydrate, fat and protein oxidized are determined primarily by the amount and composition of the food consumed (Flatt, 1977). Flatt (1977) has presented a theory of energy balance based on manipulation of the RQ. He states that the fuel mix oxidized over 24 hours must be the same as that of the food ingested, for body energy content and body composition to remain at a steady state. Thus, by increasing the fat content of the diet, this would decrease the RQ of the food (Food Q). The body responds by decreasing the gaseous RQ (increasing fat oxidation). Exercise will have the same effect. But, it may be difficult to achieve an average RQ as low as the

Food RQ on a high fat diet, if physical activity is not routinely done. On the present study, subjects were sedentary. Thus, it is likely that the RQ averaged over a 24-hour period would be greater than the Food Q when subjects were fed the high fat diets, which would promote the maintenance of body weight more than the high carbohydrate diets, as was seen when caloric intake was decreased. The long-term effects of a high fat diet may ultimately affect body composition, due to efficient conversion of dietary fat to body fat and decreased energy expenditure.

The present study suggests that decreasing the Cho:Fat ratio, and thereby increasing the fat content of the diet over a two-week period, influences the fuel mix oxidized by the body. A high fat diet may result in decreased energy expenditure, but longer measurements of energy expenditure are needed to assess the full significance of this. Although speculative at this point, the proportion of carbohydrate and fat in the diet may play a major role in energy metabolism and storage.

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Chapter 5.
Influence of Dietary Carbohydrate to Fat Ratio on Whole Body Nitrogen Retention and Body Composition in Adult Rats

Introduction

Protein intake and energy intake independently affect nitrogen metabolism (Munro, 1964; Fuller and Crofts, 1977; Young et al., 1975; Waterlow, 1968; Golden et al., 1977). However, there is disagreement as to whether or not the non-protein source of dietary energy influences nitrogen retention. In animals, feeding a high carbohydrate diet (Thomson and Munro, 1955) or a high fat diet (Forbes et al., 1946a,b; Rand et al., 1958) may improve nitrogen balance. Also, both treatments have been reported to result in an equivalent effect on nitrogen balance (Carew and Hill, 1964). In humans given oral or intravenous feedings, a high carbohydrate diet (Richardson et al., 1979; Long et al., 1977), a high fat diet (Pineault et al., 1985; Macfie et al., 1981), or both when investigated simultaneously, increase nitrogen balance (Bark et al., 1976; Nordenstrom et al., 1983). These diet treatments represent many different ratios of carbohydrate and fat, which makes their comparison difficult and is presumably one reason for the controversial results.

In a previous study, we observed greater nitrogen balance in healthy adult male subjects fed a high fat diet, providing a dietary carbohydrate to fat ratio of 1:1, compared to a high carbohydrate diet (Cho:Fat 2:1) at isonitrogenous, isoenergetic intakes (Chapter 3). Leveille and Cloutier (1987) have concluded that the composition of the diet with respect to the proportion of major nutrients — protein, fat and carbohydrate — also has a profound and variable effect on the efficiency of utilization of food energy. Diet composition

• A version of Chapter 5 has been submitted for publication:

McCargar, L.J., Baracos, V.E. and Clandinin, M.T. Influence of dietary carbohydrate to fat ratio on whole body nitrogen retention in adult rats (submitted, J. Nutr. 1988).

may influence the availability of energy for physical work, for tissue storage or for physiological functions (Leveille and Cloutier, 1987), therefore altering body composition.

The purpose of this study was to assess the effect of diet composition on nitrogen balance and body composition of adult rats by providing diets of constant energy and constant protein content over a wide range of carbohydrate and fat ratios for a six-week time period.

Materials and Methods

Animals and experimental design

Forty-two adult male Sprague-Dawley rats (Charles River, St. Constant, Quebec), weighing 295 ± 9 g were used. Six animals weighing 297 ± 10 g were sacrificed for pre-treatment carcass analysis. The remaining rats were divided into six treatment groups containing six animals each. These animals were housed individually in metabolic cages for quantitative collection of feces and urine. Animals were kept on a 12-hour light/dark cycle at 21°C.

The rats were fed diets of constant energy and essential nutrients for six weeks. The diets differed only in the ratio of carbohydrate to fat (Cho:Fat), and ranged from a ratio of 0.5 to 3.0 (Table 5.1). Dietary protein was supplied in all diets at a calculated level of 22% of total metabolizable energy. Food intake was measured daily throughout the study. The caloric density of the diets decreased as the fat content decreased between the ratios of 0.5 and 3.0 (i.e., 4.51, 4.15, 3.96, 3.85, 3.77 and 3.71 kcal/g). To ensure equal daily nutrient intakes, the diets were provided to the animals in increasing amounts between those fed the ratios of 0.5 and 3.0 (i.e., 20.0, 21.8, 22.8, 23.4, 24.0 and 24.4 g/d). Food spillage was also monitored. Water was provided ad libitum. Animals were weighed every four days. During the final eight days, 48-hour

Table 5.1
Experimental diets

Composition	Cho:Fat Ratio *(% kcal)*					
	0.5	1.0	1.5	2.0	2.5	3.0
	g/kg of total diet					
casein[1]	287	264	252	245	239	235
fat[2]	260	179	136	111	93	80
carbohydrate[3]	301.5	417.96	479.02	514.47	541.72	560.78
cellulose[4]	80	73.4	70.2	68.4	66.7	65.6
vitamin mix[5]	10	9.2	8.8	8.6	8.3	8.2
mineral mix[6]	50	45.9	43.9	42.7	41.7	41.0
choline[7]	2.75	2.52	2.41	2.35	2.29	2.25
i-inositol[8]	6.25	5.73	5.48	5.34	5.21	5.12
l-methionine[9]	2.50	2.29	2.19	2.14	2.08	2.05
	% of kcal					
Protein	22	22	22	22	22	22
Fat	52	39	31	26	22	19
Carbohydrate	26	39	47	52	56	59

1 high protein casein, Teklad Test Diets, Madison, WI.
2 hydrogenated beef tallow; soyabean oil (P:S 0.2) Canada Packers, Edmonton, AB.
3 dextrose-technical monhydrate, Canada Packers; cornstarch, St. Lawrence Cornstarch Ltd., Mississauga, ON (1:1).
4 non-nutritive fiber, Teklad.
5 AOAC, Teklad.
6 Bernhart-Tomarelli, Teklad.
7 choline chloride, Teklad.
8 i-inositol, Teklad.
9 l-methionine NRC, Teklad.

urine and fecal samples were collected. Urine, preserved with dilute (0.5N) H_2SO_4, and feces were frozen at -30°C until further analysis. The study was approved by the Animal Policy and Welfare Committee, University of Alberta.

Analytical procedures

Rats were sacrificed using diethyl ether. The contents of the gastrointestinal tract were removed and discarded. Carcasses were frozen and then prepared for analysis with modifications to a previously reported method (Hartsook and Hershberger, 1963). The hair of the rat has previously been shown to cause large variations in carcass nitrogen determination (Crews et. al, 1969). Thus, each animal was partially thawed and then shaved to eliminate this source of variation. To facilitate complete homogenization, each animal carcass was autoclaved for six hours at 16 pounds pressure at 120°C. The carcass was cooled, 400 ml water was added, and the carcass homogenized in a Waring blender. Aliquots of the homogenates were frozen for nitrogen analysis and were also freeze-dried for fat analysis. Nitrogen content of urine, feces, and the carcass was determined by a modified Kjeldahl method (Kjeltec System, Tecator, Hoganas, Sweden). Carcass fat was measured by petroleum ether extraction over nine hours, using a Goldfisch apparatus (AOAC, 1980). Duplicate aliquots were analyzed for each procedure.

All data was analyzed by analysis of variance procedures (Steel and Torrie, 1980). Individual treatments were compared using a Duncan's multiple range test (Steel and Torrie, 1980). Linear regression analysis was conducted on all data. Values were considered different at a significance level of $p < 0.05$.

Results

Over the six-week feeding period, the calculated mean energy intake was 93 ± 0.82 kcal/d and protein intake was 5.3 ± 0.07 g/d. Energy intakes resulted in a range between treatments of only 6 kcal/d (Table 5.2; F = 2.02, NS). However, the lowest and highest energy intakes were different when analyzed by a Duncan's multiple range test (dietary Cho:Fat ratio of 1.5 compared with 2.0). Protein intakes were not significantly different between treatment groups (Table 5.2). There was no difference in initial body weights of the groups of animals. Final body weights, however, were different between animals fed diets providing a Cho:Fat ratio of 0.5 versus 3.0 ($p < 0.05$) and a linear response was observed (Figure 5.1) such that as the carbohydrate content of the diet increased, the body weight increased ($r = .92$). Rats on the highest fat diet gained 150 g over the period of study, while rats on the highest carbohydrate diet gained 200 g.

Table 5.2
Energy and protein intakes of rats fed diets providing different ratios of carbohydrate to fat

	Cho:Fat Ratio					
	0.5	1.0	1.5	2.0	2.5	3.0
Energy intake* (kcal/d)	93 ± 2†ab	94 ± 1ab	90 ± 1b	96 ± 1a	93 ± 1ab	92 ± 1ab
Protein intake‡ (g/d)	5.5 ± .1	5.6 ± .1	5.1 ± .2	5.3 ± .3	5.3 ± .2	5.3 ± .2

n = 6 per group
* Calculated values based on Atwater factors.
† mean ± SEM .
a Values not sharing a common superscript are significantly different ($p < 0.05$).
‡ NS.

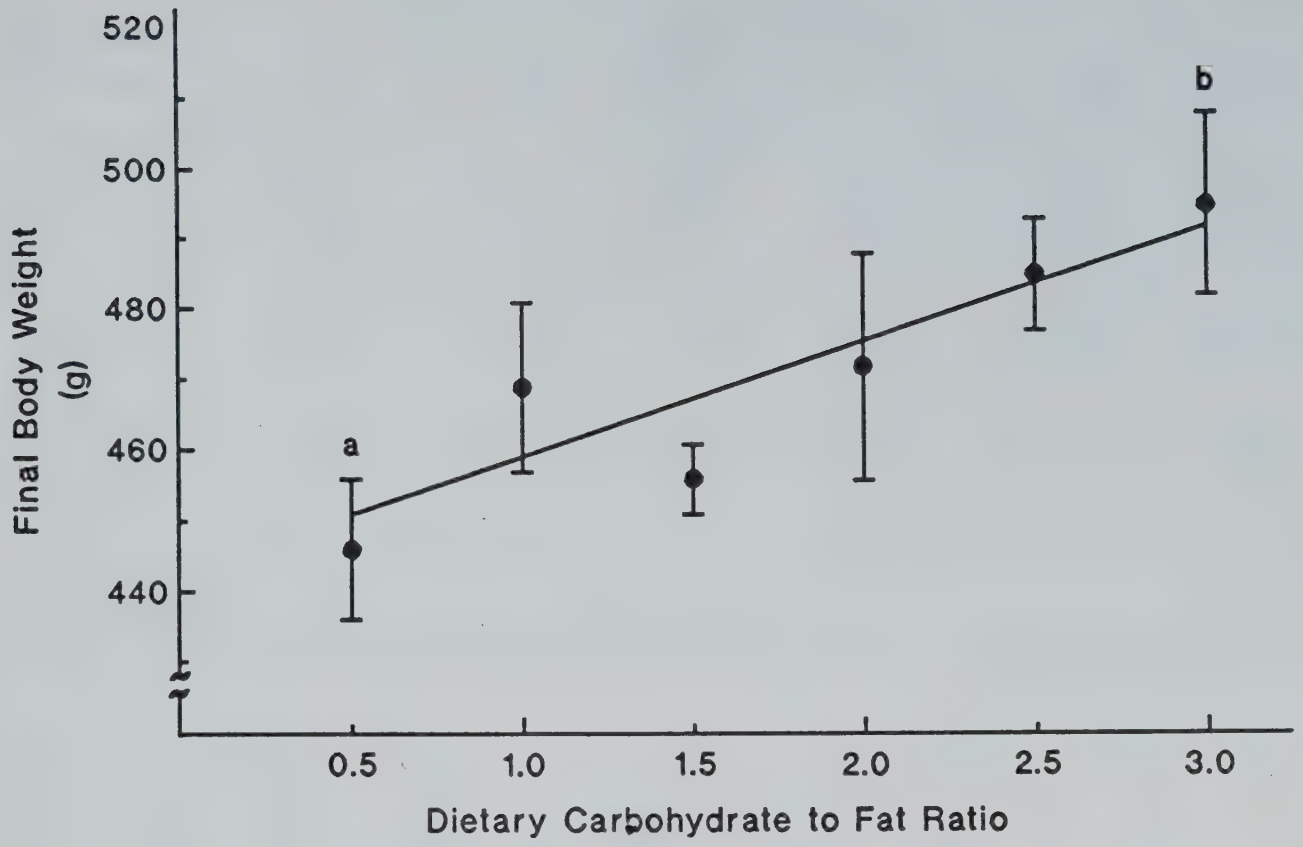

Figure 5.1 Final body weight of rats fed diets providing different ratios of carbohydrate to fat for six weeks.
The slope of the line is y = 439.4 + 17.66 x; r = .92. The group fed the diet with the Cho:Fat ratio of 0.5 is different from the diet group fed the Cho:Fat ratio of 3.0 ($p < 0.05$) (a vs. b).

Nitrogen balance data indicated a consistent negative regression relationship (r = -.96) between nitrogen balance and decreased fat in the diet, but only over the range of dietary Cho:Fat ratio of 0.5 to 2.0. Animals fed diets providing a Cho:Fat ratio of 2.5 or greater showed a trend towards increasing nitrogen balance compared to Cho:Fat of 2.0 (Table 5.3) when accretion was expressed in terms of absolute values (mg/d), per gram of final body weight, or per kcal energy gained (Figure 5.2).

Table 5.3
Nitrogen balance of rats fed diets providing different ratios of carbohydrate to fat

Nitrogen balance	Cho:Fat Ratio					
	0.5	1.0	1.5	2.0	2.5	3.0
mg/d	242.3* ± 16.4	237.2 ± 21.1	225.8 ± 31.1	204.7 ± 11.4	251.2 ± 26.5	240.8 ± 16.6
mg/g body weight	.542 ± .029	.506 ± .043	.496 ± .069	.433 ± .015	.516 ± .050	.485 ± .027
mg/kcal of energy gained	.81 ± .08[a]	.56 ± .05[ab]	.63 ± .11[ab]	.37 ± .03[b]	.47 ± .06[b]	.55 ± .05[ab]

n = 6 per group
* Mean ± SEM.
[a] Values not sharing a common superscript letter are significantly different ($p < 0.05$).

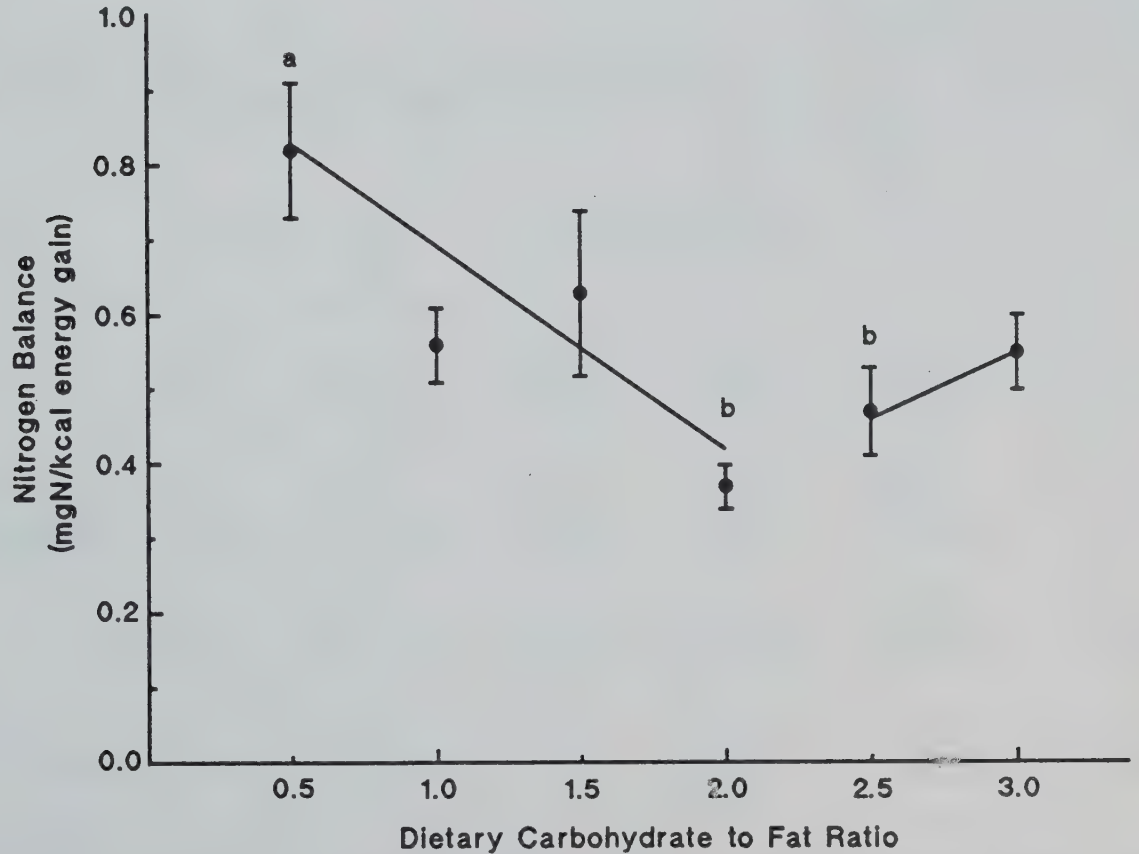

Figure 5.2 Nitrogen balance per kcal energy gain in rats fed diets providing different ratios of carbohydrate to fat for six weeks. The group fed the diet with the Cho:Fat ratio of 0.5 is different from the diet group fed the Cho:Fat ratios of 2.0 and 2.5 (a vs b = $p < 0.05$).

Body composition measurements of carcass protein and carcass fat content (Table 5.4) varied between animal groups because of differences in final body weight. When values were corrected for final body weight, however, some differences in body composition were still noted. Carcass fat content showed considerable within-group variation, especially in the animals with the highest mean values for body fat. Animals fed the diet providing a Cho:Fat ratio of 2.0 had the lowest carcass protein and highest carcass fat content as a percent of body weight ($p < 0.05$). Animals fed the 0.5 Cho:Fat ratio diet exhibited the lowest carcass fat and the highest carcass protein content (% of body weight) ($p < 0.05$). The latter value, however, was equally as high in the 1.5 and 3.0 ratio diet groups. Again, as was the case for nitrogen balance data, animals fed diets providing a Cho:Fat ratio of 2.5 or greater showed a

Table 5.4
Body composition of rats fed diets providing different ratios of carbohydrate to fat

Carcass content	Cho:Fat Ratio 0.5	1.0	1.5	2.0	2.5	3.0
protein (g)	89.71* ± 2.33^{ab}	94.18 ± 1.84^{abc}	94.09 ± 1.49^{abc}	87.69 ± 2.67^{a}	96.62 ± 1.38^{bc}	101.69 ± 2.11^{c}
protein (% of body weight)	21.67 ± .23^{a}	21.20 ± .20^{ab}	21.78 ± .20^{a}	19.73 ± .93^{b}	20.82 ± .39^{ab}	21.53 ± .29^{a}
fat (g)	36.74 ± 2.68^{a}	48.70 ± 4.12^{ab}	41.74 ± 2.68^{ab}	62.43 ± 8.81^{ab}	62.57 ± 6.86^{b}	49.58 ± 7.25^{ab}
fat (% of body weight)	9.91 ± .71^{a}	10.86 ± .69^{ab}	9.67 ± .62^{ab}	13.78 ± 1.61^{b}	13.38 ± 1.33^{ab}	10.39 ± 1.40^{ab}

n = 6 per group
Animals sacrificed at beginning of experiment had the following body composition: 55.99 ± 1.27 g protein (20.37 ± .27% of body weight) and 17.6 g fat (6.40 ± .47% of body weight.
* Mean ± SEM.
a Values not sharing a common superscript letter are significantly different ($p < 0.05$).

trend towards increasing protein gain expressed as grams or as a percentage of body weight. Carcass protein and fat content are illustrated as a percentage of energy gain (i.e, kcal protein energy gained ÷ total energy gained x 100) (Figure 5.3). Total energy gain and energy gain as fat were lowest in the group fed the diet providing a Cho:Fat ratio of 0.5 and highest in the group fed the diet providing a Cho:Fat ratio of 2.0 ($p < 0.05$). The proportion of energy gain as protein relative to energy intake was highest in the group fed the diet providing a Cho:Fat ratio of 0.5. This was twice as much as the lowest gain observed in the group fed the diet providing a Cho:Fat ratio of 2.0 ($p > 0.05$). Diet composition thus appeared to be responsible for a number of metabolic changes in these animals fed over a six-week study period.

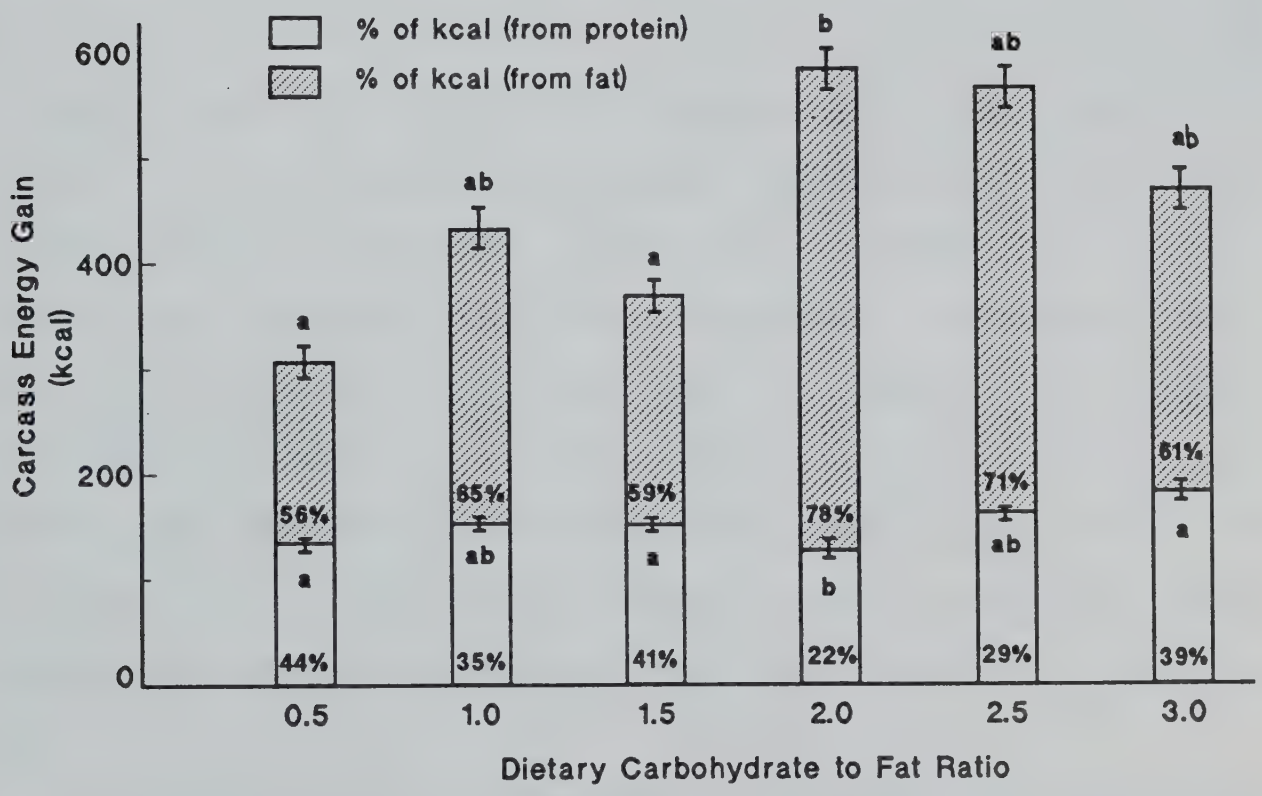

Figure 5.3 Percent of energy gain as protein calories or fat calories.
Values with different superscripts are significantly different ($p < 0.05$).

Discussion

In the present study, body weight gain increased as the proportion of carbohydrate in the diet increased. Two diet ratios resulted in the extremes in nitrogen and fat indices in relation to energy gain, even though gross energy intakes were constant. For example, feeding the high fat diet (Cho:Fat 0.5) resulted in the highest nitrogen balance (mg N/kcal energy gain), the highest proportion of gain as protein (as a % of energy gain), and the lowest proportion of gain as fat (as a % of energy gain). The opposite was observed for the animals fed the Cho:Fat ratio of 2.0.

In the present study, altering the proportion of carbohydrate to fat in the diet resulted in differences in body weight, nitrogen balance and body composition in adult rats. The diets fed were more than adequate in protein (requirement = 200 mgN/Wkg$^{.75}$) and energy (requirement = 106 kcal ME/Wkg$^{.75}$), based on maintenance requirements (NRC, 1978). The rat will generally modify food consumption in relation to its energy requirement (Kleiber, 1975), with a proportionate increase in consumption in relation to total nutrient dilution. This regulation of food intake to maintain energy intake is consistent with the present study, where mean intakes increased from 20.6 – 24.9 g/d as the nutrient density decreased. In research involving dietary manipulation and protein or energy metabolism, it is essential that the nutrient-to-energy ratio of the diet and the total caloric intake be controlled (Leveille and Cloutier, 1987). Studies where ad libitum feeding is allowed add another variable to be considered (Salmon and Flatt, 1985; Schreiber and Elvehjem, 1955).

Hartsook et al. (1973) investigated a group of animals on a similar protocol to the present study. When protein was fed at 22% of energy, nitrogen gain (as a percent of food nitrogen) was higher (28.5%) with a high fat diet

(Cho:Fat 0.9) than with a high carbohydrate diet (22.9% – Cho:Fat 2.0). Also consistent with the present data, fat gain was slightly lower as the dietary carbohydrate to fat ratio decreased (i.e., as the fat in the diet increased).

During growth, the amount of energy deposited as protein is relatively small compared to that deposited as fat or dissipated as heat (Pullar and Webster, 1977). This is even more so in mature animals. Adult rats were chosen for study because they are at a more steady state of development than weanling rats. It appears that growth, however, still remains linear in Sprague-Dawley rats until they reach approximately 500 grams or an age of 16 weeks (NRC, 1978). The greatest difference in the animals studied was in carcass fat deposition. The cost of conversion of dietary carbohydrate to fat is about 24% (Flatt, 1977) to 28% (Leveille and Cloutier, 1987) of the energy of the substrate. Feeding a high carbohydrate diet results in increased lipogenesis, and is positively correlated with energy expenditure (Acheson et al., 1984). Converting dietary fat to body fat is a more efficient process, requiring approximately 2 – 7% of the energy of the substrate for the conversion (Flatt, 1977; Heim, 1981; Leveille and Cloutier, 1987). Thus, it may be inferred that feeding a high carbohydrate compared to a high fat diet may result in increased energy expenditure, especially if animals are near their mature carcass nitrogen deposition. It is generally agreed that in a species such as the rat, there is a requirement of 12.6 kcal ME/g fat deposited if the diet consists predominantly of carbohydrate, and 10.4 kcal ME/g fat deposited if the diet is rich in triglyceride (ARC/MRC Committee, 1974). The energy cost for protein is 12.7 kcal ME/g of protein deposited (Pullar and Webster, 1977). Based on these figures, it would appear that the animals expended more energy for tissue growth and storage as the carbohydrate to fat ratio of the diet increased, because fat gains increased.

The question of the effect of diet composition on energy balance must be investigated further. Chemical energy in food is converted to work, heat or stored energy (Leveille and Cloutier, 1987). When feeding the diets of different carbohydrate to fat ratios, energy intake was similar. Although metabolizable energy decreases with increased fat, it is unlikely that this is solely responsible for the differences in energy gain. The thermic effect of feeding is slightly higher when consuming high carbohydrate diets compared to high fat diets (Flatt, 1977). It is expected that the thermic effect of exercise was similar between groups. Still, more energy was stored by animals fed the high carbohydrate diet compared to the high fat diet. It is difficult to account for the lower weight gains of the high fat diet groups. Excess available energy may in some way have stimulated mechanisms of substrate cycling and dissipated increased amounts of energy as heat.

Perhaps the available energy in the high fat diet groups was used for metabolic work. Protein synthesis is an energy-requiring process, which is supported by a pool of energy-yielding metabolites of carbohydrate and fat. It is thought that the differences in the energetic properties of carbohydrate and fat is one way in which protein metabolism is affected (Munro, 1951). The energy cost of protein synthesis is estimated to account for about 20% of oxygen consumption (Reeds and Garlick, 1984), and a change in the rate of protein synthesis is a major factor determining changes in metabolic rate (Garrow, 1985). The high fat diet may have resulted in a change in protein synthesis rate, requiring increased energy. This would not be detectable from nitrogen balance data.

Protein-sparing occurs when nitrogen losses are minimized. Urinary nitrogen is the largest source of nitrogen losses (Lentner, 1981), composed primarily of urea and ammonia. These losses in nitrogen result from the

deamination of amino acids which are oxidized or used for gluconeogenesis. It has been shown that increased concentrations of fatty acids in the blood increase the rate of fatty acid oxidation in the muscle, which results in decreased glucose utilization and oxidation (Newsholme and Leech, 1986). Thus, if fatty acids are preferentially oxidized and glucose oxidation is inhibited, it is inferred that amino acid oxidation would also be inhibited. Fatty acids are mobilized under conditions of "carbohydrate stress" when the glycogen store in the liver is depleted (Newsholme and Leech, 1986). Such a stress might be sustained exercise, starvation or a low carbohydrate (high fat) diet. Plasma ketone levels also increase in these situations. There is some evidence that high concentrations of ketones inhibit the rate of protein degradation in muscle, and therefore results in protein-sparing (Newsholme and Leech, 1986; Wu and Thompson, 1987). Thus, when feeding a high fat diet, amino acid oxidation and protein degradation may decrease, and because fatty acid oxidation increases, less fatty acids would be stored as triglyceride.

The diet ratios of 2.5 and 3.0 led to results in the opposite direction of the other four diet groups for nitrogen balance and body composition measurements. When feeding these two diets where carbohydrate was supplied as 56% and 59% of energy respectively, the effects of insulin may dominate and thereby increase glucose utilization, decrease lipolysis (Newsholme and Leech, 1986) and increase amino acid uptake into muscle (Munro, 1964). Although insulin is always present in the blood, there may be a concentration threshold at which these metabolic processes are regulated. The animals fed the diets providing a Cho:Fat ratio of 2.5 and 3.0 exhibited the highest body weights, even though carcass fat was highest in the animals fed the diet providing a Cho:Fat ratio of 2.0. High carbohydrate diets result in

increased storage of glycogen which is 65% water (Newsholme and Leech, 1986). Body water content was higher in animals consuming the higher carbohydrate intakes in this and other studies (Macfie et al., 1981, Shulman et al., 1984). It is conceivable that this could account for the remainder of the weight gain.

Alterations in diet composition resulted in changes in metabolic processes. Whether these changes are components of energy metabolism, such as rate of heat production and/or protein metabolism measured as rates of protein synthesis or amino acid oxidation, should be investigated.

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Chapter 6.
Evaluation of Energy Metabolism of Rats fed Diets with High and Low Ratios of Carbohydrate to Fat

Introduction

Recently, there has been a renewed interest in the influence of diet composition on energy metabolism (Donato and Hegsted, 1985; Donato, 1987; Bray, 1987; Flatt, 1987; Leveille and Cloutier, 1987). The focus has switched from the concept of energy balance to that of individual nutrient balances of carbohydrate, protein and fat (Bray, 1987; Flatt, 1987). It appears that changing the macro-nutrient mix in the diet can also influence the efficiency with which dietary energy is utilized (Donato and Hegsted, 1985; Donato, 1987; Leveille and Cloutier, 1987).

In adult rats, Forbes et al. (1946) observed increased efficiency of energy retention as fat in the diet increased from 2 to 30% by weight, with gross energy and protein content kept constant. Carcass energy increased as fat intake increased. Weanling rats fed a high fat diet ad libitum compared to a low fat diet showed an increased rate of weight gain and energy retention (Mickelson et al., 1955). When supplements of sucrose, protein or fat were supplied at graded levels with a nutritionally balanced basal diet, animals in the fat-supplemented group had the greatest body fat deposition, and dietary fat showed a greater efficiency of utilization than sucrose or protein (Donato and Hegsted, 1985).

We have observations that contradict these reports (Chapter 5). That is, weight gain in adult rats increased as the proportion of carbohydrate in the diet increased ($r = 0.92$) over a wide range of carbohydrate to fat ratios (0.5 - 3.0). The lowest and highest values for fat deposition (as a percent of energy gain) were observed in animals fed the diet providing a carbohydrate to fat ratio of

0.5 (Cho:Fat 0.5) and 2.0 (Cho:Fat 2.0) respectively. Because of these differences, it was necessary to further examine the effect of the two diets on energy metabolism. The model for the partitioning of food energy (Figure 6.1) by Leveille and Cloutier (1987) was used as the theoretical basis for evaluation of the two diet treatments.

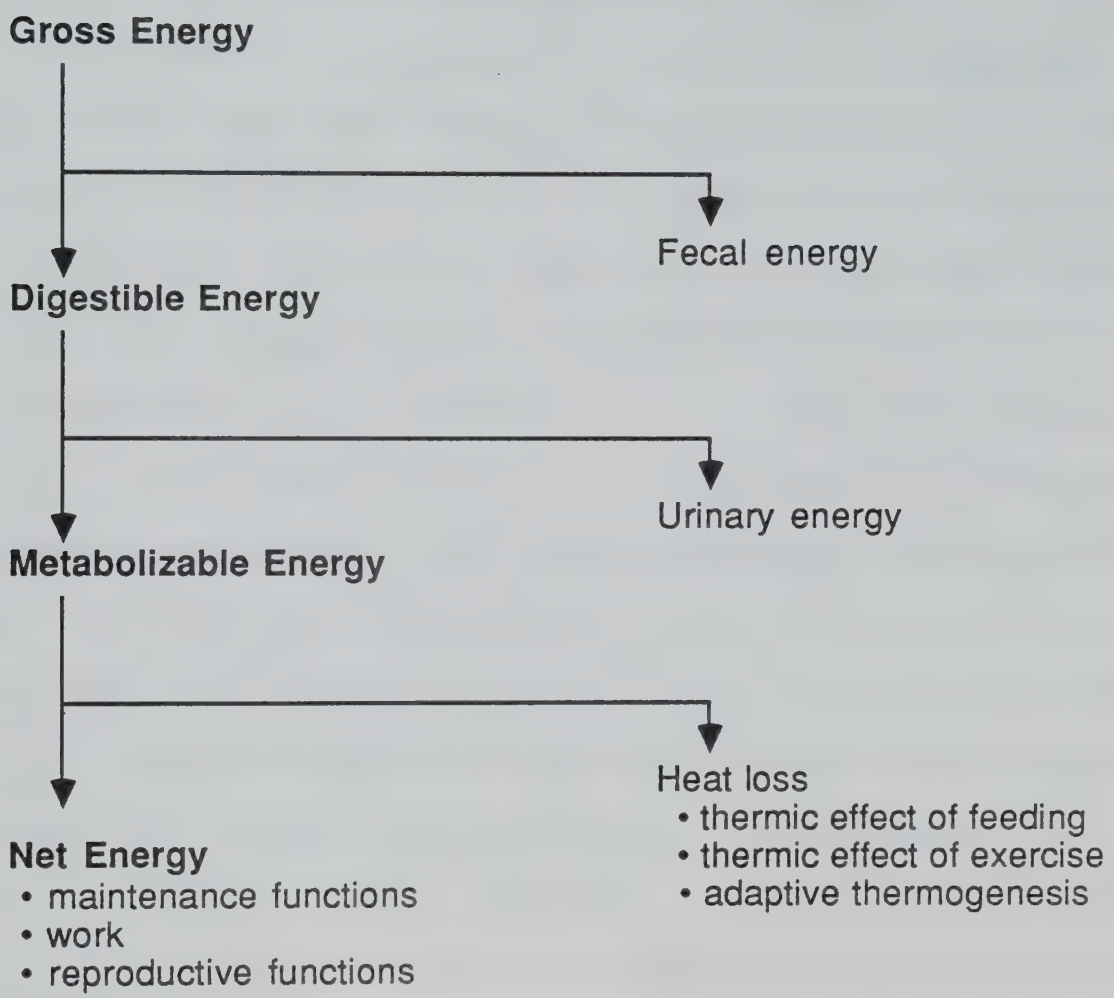

Figure 6.1 Classification of energy in food.
(adapted from Leveille and Cloutier, 1987)

Materials and Methods

Animals and experimental design

Twenty-four adult male Sprague-Dawley rats obtained from Biological Sciences Animal Services, University of Alberta, Edmonton, weighing 319 ± 21 g, were used. Eight animals were sacrificed for pre-treatment carcass analysis. The remaining rats were divided into two treatment groups of eight animals each. These animals were housed individually in metabolic cages for quantitative collection of feces and urine in an environmental walk-in chamber. Animals were kept on a 12-hour light/dark cycle at 21 - 24°C.

For six weeks, rats were fed diets in which gross energy, protein and essential nutrients were kept constant. The diets differed only in the ratio of dietary carbohydrate to fat (0.5 and 2.0) as a percentage of total energy (Table 6.1). Dietary protein was supplied in both diets at a calculated level of 22% of total metabolizable energy. Because of the different fat content in the diets, the caloric densities were different (Cho:Fat 0.5 = 5.43 kcal gross energy/g diet; Cho:Fat 2.0 = 4.44 kcal/g), determined by bomb calorimetry (Model #1241, Parr Instrument Co., Moline, IL). Eight measurements were done for each batch of diet. Thus, the diet providing a Cho:Fat ratio of 2.0 was provided to the animals in amounts multiplied by a factor of 1.22 of the intake of the Cho:Fat ratio of 0.5 diet group to ensure equal nutrient intakes. Food intake was measured every two days, and food spillage was monitored. Water was provided ad libitum. Indirect calorimetry was used to assess energy expenditures of 8 animals initially (4 from each treatment group) and of all 16 animals at the end of the six-week study period. Twenty-four hour measurements of oxygen consumption (VO_2) were obtained. Heat loss values were also determined on 4 animals (2/group) at the end of the diet treatments by direct calorimetry.

Table 6.1
Composition of experimental diets

Composition	Cho:Fat Ratio *(% kcal)* *	
	0.5	2.0
	g/kg of total diet	
casein[1]	287	245
fat[2]	260	111
carbohydrate[3]	301.5	514.47
cellulose[4]	80	68.4
vitamin mix[5]	10	8.6
mineral mix[6]	50	42.7
choline[7]	2.75	2.35
i-inositol[8]	6.25	5.3
l-methionine[9]	2.50	2.14
	% of kcal	
Protein	22	22
Fat	52	26
Carbohydrate	26	52

* The carbohydrate to fat ratio was determined by: [carbohydrate in the diet (% of total energy ÷ fat in the diet] (% of total energy).

1 high protein casein, Teklad Test Diets, Madison, WI.

2 hydrogenated beef tallow; soyabean oil (P:S 0.2) Canada Packers, Edmonton, AB. The fat in the diets was 75% hydrogenated beef tallow containing approximately 25% 16:0 and 50% 18:0 fatty acids; and 25% soyabean oil containing approximately 22% 18:1 and 50% 18:2 fatty acids.

3 dextrose-technical monohydrate, Canada Packers; cornstarch, St. Lawrence Cornstarch Ltd., Mississauga, ON (1:1).

4 non-nutritive fiber, Teklad.

5 AOAC, Teklad.

6 Bernhart-Tomarelli, Teklad.

7 choline chloride, Teklad.

8 i-inositol, Teklad.

9 l-methionine NRC, Teklad.

The animals were tested approximately 5 - 6 h after the dark period on the last day of the six weeks of feeding. Rats will eat most of their food during the dark period (Ip et al., 1977). Thus, the animals were in a post-absorptive state.

For determination of O_2 consumption and CO_2 production, animals were put into a small plexiglass chamber. Exhaust gas from the metabolic chamber was divided into two streams. One stream was for measurement of VO_2 by a S-3A/ii oxygen analyzer (Applied Electro Chemistry, Sunnyvale, CA) following drying (Drierite) and CO_2 removal by Ascarite. The second stream of air was dried (Drierite) for measurement of VCO_2 by a CO_2 analyzer (model CD/3A, Applied Electro Chemistry, Sunnyvale, CA). A computer-based (IBM) data-acquisition system was used to calculate VO_2, VCO_2 and RQ. Heat production was calculated using Kleiber's equation (Kleiber, 1975):

$$HP\ (kcal/h) = VO_2\ (1/h) \times [(87 + 27\ RQ)/22.4] \quad \text{(Appendix III)}$$

Measurements of heat loss were determined by heat transfer across the wall of a whole body calorimeter (C-Beck Envelope, Thermonetics Corp., San Diego, CA).

Animals were weighed weekly. During the final 8 days, 48-hour urine and fecal samples were collected. Urine, preserved with dilute (0.5 N) H_2SO_4, and feces were frozen at -30°C until further analysis. The study was approved by the Animal Policy and Welfare Committee, University of Alberta.

Analytical procedures

Rats were sacrificed using CO_2 for euthanasia. The contents of the gastrointestinal tract were removed and discarded. Carcasses were frozen and then prepared for analysis with modifications to a previously reported method (Hartsook and Hershberger, 1963; Chapter 5). To facilitate complete

homogenization, each animal carcass was autoclaved for six hours at 16 pounds pressure at 120°C. The carcass was cooled, 400 ml water was added, and the carcass homogenized in a Waring blender. Aliquots of the homogenates were freeze-dried. Energy content of urine, feces, and carcass was determined by bomb calorimetry. For each procedure, duplicate aliquots were analyzed.

The results of the two treatments were analyzed by t-test statistics (Steel and Torrie, 1980).

Results

Gross energy intake

Food intake was adjusted according to the group that ate the least amount of diet (g/d) (Cho:Fat 0.5 diet group), but very little manipulation was required. The gross energy intakes of the two groups were not different when the actual weight of diet intake was multiplied by the caloric density of each diet (Table 6.2).

Table 6.2
Gross energy intakes of rats fed diets providing different ratios of carbohydrate to fat

	Cho:Fat Ratio	
	0.5*	2.0*
Energy intake (kcal/d)	120.5 ± 1.2†	119.6 ± 1.1
Food intake (g/d)	22.2 ± 0.2	27.0 ± 0.3

n = 8 per group
* Caloric density of each diet is 5.43 kcal/g and 4.44 kcal/g for the Cho:Fat ratios of 0.5 and 2.0 respectively.
† Mean ± SEM.

Energy output

Dietary energy is not completely available to the animal because losses occur in the feces and urine (Table 6.3). Fecal energy losses were significantly greater ($p < 0.001$) for the high fat diet group (Cho:Fat 0.5) which showed a low digestibility of the diet (69 ± 0.5%) compared to the high carbohydrate diet group (Cho:Fat 2.0) with a digestibility of 83 ± 0.5%. Thus, digestible energy for the diet groups was 82.7 kcal/d (Cho:Fat 0.5) and 98.9 kcal/d (Cho:Fat 2.0). Mean urinary energy losses were similar between both diet groups due to considerable within-group variation, shown by the standard errors. Metabolizable energy for the diet groups was 80.1 kcal/d (Cho:Fat 0.5) and 95.6 kcal/d (Cho:Fat 2.0).

Table 6.3
Energy losses in feces and urine of rats fed diets providing different ratios of carbohydrate to fat

	Cho:Fat Ratio	
	0.5	2.0
Fecal losses:		
weight (g/d)	6.28 ± .24*†	4.11 ± .20
energy (kcal/g)	6.02 ± .03†	5.05 ± .03
energy (kcal/d)	37.77 ± 1.36†	20.75 ± .98
Urinary losses:		
energy (kcal/g)‡	0.92 ± .12	1.06 ± .19
energy (kcal/d)	2.64 ± .43	3.26 ± .68

n = 8 per group
* Mean ± SEM.
† Different from 2.0 ratio ($p < 0.001$).
‡ Value is per g urinary dry matter.

Heat production was determined over a 24-hour period, based on actual measurements of O_2 consumption and CO_2 production. Energy expenditure (kcal/rat/h) was higher ($0 < 0.001$) in animals fed the high carbohydrate diet versus the high fat diet (Figure 6.2). When corrected for body weight (kcal/g/h), a difference ($p < 0.001$) was still observed between groups (Figure 6.3), but this was due to the differences during the final 12 hours of measurement. The total of the hourly values resulted in mean daily resting energy expenditures of 55.4 kcal/d and 66.2 kcal/d for the high fat and high carbohydrate diet groups respectively.

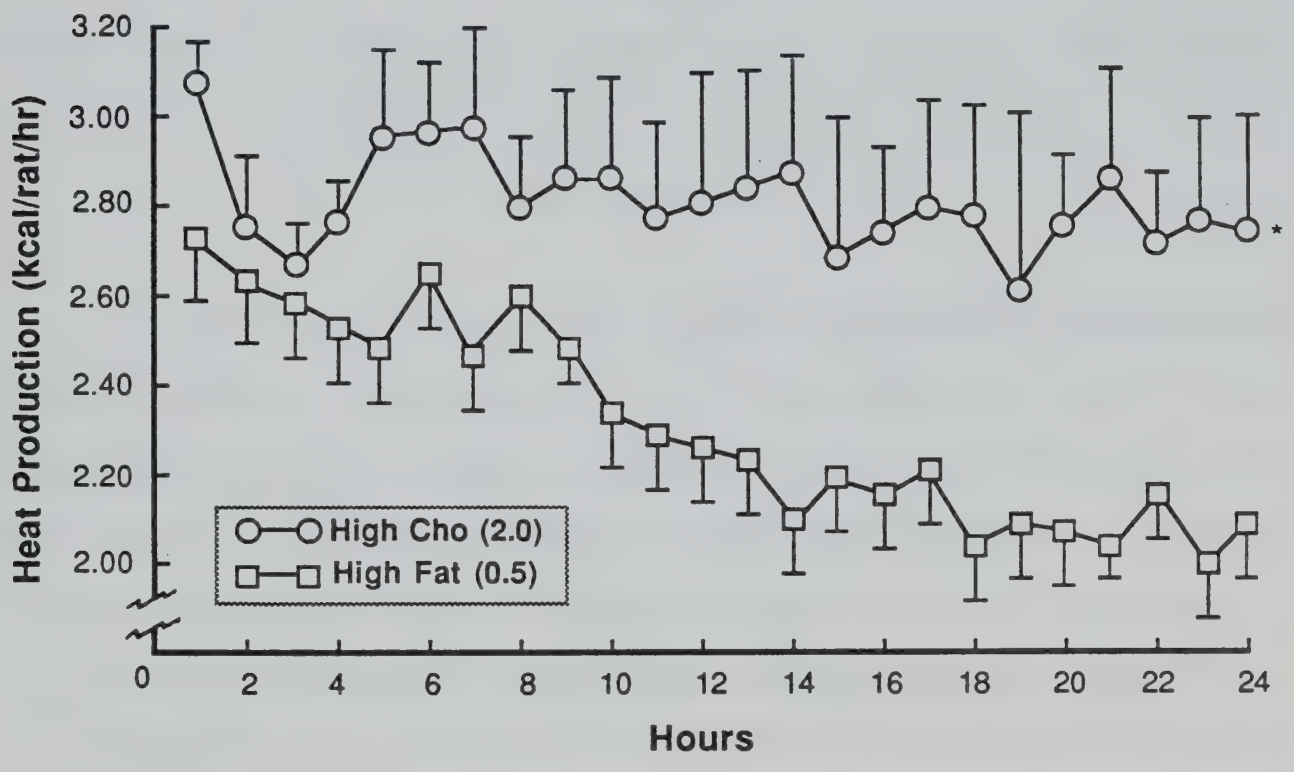

Figure 6.2 Twenty-four hour heat production (kcal/rat/hr) of rats fed diets providing different ratios of carbohydrate to fat.
High Fat (0.5) = Dietary carbohydrate to fat ratio of 0.5:1.
High Cho (2.0) = Dietary carbohydrate to fat ratio of 2.0:1.
* Different from high fat diet (0.5) $p < 0.001$.

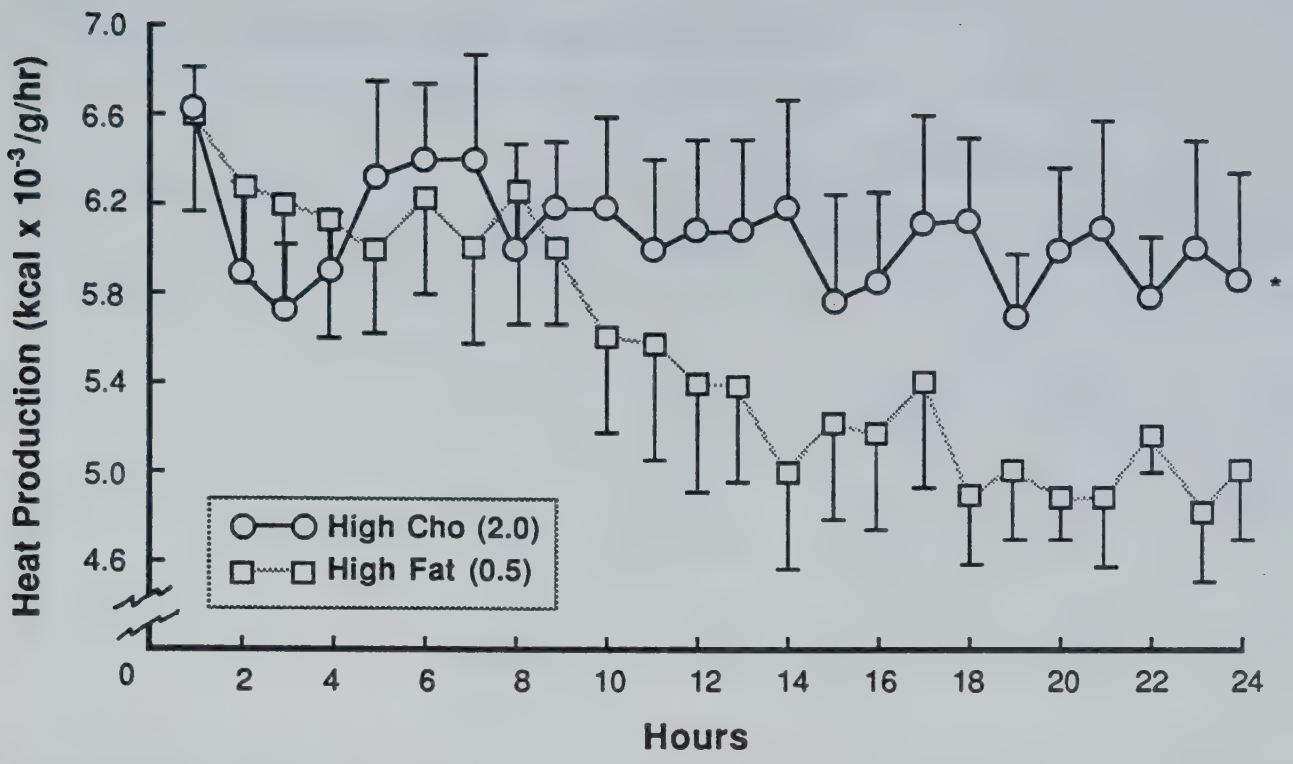

Figure 6.3 Twenty-four hour heat production (kcal/g/hr) of rats fed diets providing different ratios of carbohydrate to fat.
High Fat (0.5) = Dietary carbohydrate to fat ratio of 0.5:1.
High Cho (2.0) = Dietary carbohydrate to fat ratio of 2.0:1.
* Different from high fat diet (0.5) $p < 0.001$.

The differences between initial and final heat production measurements were determined by subtracting the mean heat production value for eight chow-fed rats tested at the beginning of the study period from the actual values for the same rats after diet treatment (4 per diet group) (Table 6.4). A trend was observed where heat production increased in the group fed the Cho:Fat ratio of 2.0, except when corrected for body weight, when both groups decreased.

Heat loss measurements (direct calorimetry) showed no consistent results, although only four animals were tested. For both diet groups, one animal exhibited a higher heat loss value, and one animal had a lower heat loss value than the measurements obtained for heat production. It is likely that a greater number of measurements would be necessary to detect differences between heat production and heat loss.

Table 6.4
Change in heat production after six weeks

Heat Production		Cho:Fat Ratio			
		0.5		2.0	
	Initial*	Final	Change	Final	Change
kcal/h	2.472 † ± .117	2.233 ± .044	- .239 ± .044	2.818 ± .278	+ .346 ± .278
kcal/d	59.34 ± 2.81	53.59 ± 1.06	- 5.75 ± 1.06	67.63 ± 6.68	+ 8.29 ± 6.68
kcal/g/d	.175 ± .008	.130 ± .006	- .045 ± .006	.145 ± .012	- .030 ± .012

n = 4 per group
* Pre-treatment measurements of 8 animals that were chow-fed.
† Mean ± SEM.

Energy storage

Mean weekly body weights of the treatment groups are shown (Table 6.5). The animals fed the high carbohydrate diet gained more weight (133 ± 8 g) than those animals fed the high fat diet (94 ± 6 g). The difference in body weight between groups was significant at weeks 5 and 6 ($p < 0.05$). However, weight gain was similar when corrected for metabolizable energy (Table 6.6).

Carcass energy gain was determined by subtracting the mean carcass energy content of the 8 rats sacrificed initially from the actual carcass energy content of each of the animals. Energy gain was greater in the high carbohydrate diet group (+664.5 ± 25.0 kcal) than in the high fat diet group (+275.4 ± 31.4 kcal) ($p < 0.001$), even when corrected for metabolizable energy. Daily gains per kcal ME were 0.165 ± 0.006 and 0.082 ± 0.009 kcal ($p < 0.001$) in the high carbohydrate and high fat diet groups respectively.

Table 6.5
Body weights of rats fed diets providing different ratios of carbohydrate to fat

Cho:Fat Ratio	Week						
	0	1	2	3	4	5	6
0.5	321* ± 9	330 ± 10	346 ±7	367 ± 8	382 ± 10	401† ± 11	416† ± 12
2.0	323 ± 5	335 ± 5	354 ± 5	371 ± 4	399 ± 10	432 ± 10	456 ± 10

n = 8 per group
* Mean ± SEM.
† Different from 2.0 ratio ($p < 0.05$).

Table 6.6
Parameters of weight gain

Weight Gain	Cho:Fat Ratio	
	0.5	2.0
grams/6 weeks	94 ± 6*†	133 ± 8
grams/day	2.25 ± .15†	3.17 ± .19
grams/kcal ME‡	.028 ± .002	.033 ± .002

n = 8 per group
* Mean ± SEM.
† Different from 2.0 ratio ($p < 0.05$).
‡ ME = metabolizable energy.

Table 6.7 summarizes the distribution of food energy for the treatment groups. During the 24-h measurement of energy expenditure, no food was provided and activity was limited by the size of the chamber. Thus, the thermic effect of feeding and exercise was not considered in this calculation. Based on determinations of carcass fat and nitrogen in a previous experiment (Chapter 5), carcass energy gain was assumed to be 56% fat calories and 44% protein calories for the high fat diet group, and 78% fat calories and 22% protein calories for the high carbohydrate diet group. There appears to be a greater amount of unaccounted-for energy output (heat loss) in the animals fed the high fat diet.

Table 6.7
Distribution of daily food energy

Energy (kcal/d)	Cho:Fat Ratio 0.5	Cho:Fat Ratio 2.0
Gross energy	120.5 ± 1.2*	119.6 ± 1.1
Digestible energy	82.7 ± 1.8	98.9 ± 1.9
Metabolizable energy	80.1 ± 1.8	95.6 ± 1.9
Heat production (resting)	55.4 ± 2.2	66.2 ± 3.6
Net energy†	24.7 ± 3.0	29.4 ± 2.7
Daily energy cost‡ for:		
fat deposition	4.2	17.3
protein deposition	6.5	7.8
Unaccounted-for energy loss:	**14.0**	**4.3**

n = 8 per group
* Mean ± SEM.
† net energy = metabolizable energy – resting heat production.
‡ Calculated from body composition data Ch. 5 Figure 5.3, and values for protein deposition (2.25 kcal/kcal protein) and fat deposition (1.15 kcal/kcal fat from dietary fat) or (1.40 kcal/kcal fat from dietary carbohydrate) (Pullar and Webster, 1977). Mean values used for calculations, thus no standard errors.

Discussion

Whether diets differing in nutrient composition influence energy utilization is relatively unexplored (Hegsted, 1974). Since this statement was made, research interest in this area has increased. Experimental difficulties occur, however, because many variables affecting energy balance must be controlled to isolate the specific effects of diet composition. Comparison between diets requires that intake of energy and all essential nutrients must be the same for each group. This may require manipulation of the diet (i.e., restriction of one group), which could result in a different metabolic response than ad libitum feeding. Possible differences in "available energy" to the animals, or in activity level, must also be considered. Differences between experiments may arise due to the choice of animals (age, sex, strain, body weight), the diet components (amount and source), method of feeding (ad libitum, intravenous, force-feeding, pair-feeding) and design factors (environment, number of animals and length of feeding). In spite of these variables, one consistent finding exists in the literature. An increase in diet fat results in an increase in energy retained (Forbes et al., 1946; Mickelson et al., 1955; Donato and Hegsted, 1985). This appears to be due to a greater net efficiency of fat, which results in more available energy for obligate demands, physical work, and storage (Hill, 1985). However, when high and low fat diets were fed ad libitum to mice (Salmon and Flatt, 1985), it was suggested that the low incidence of obesity among mice fed the low fat diets may have been due to "spontaneous limitation of food intake," not because of less efficient carbohydrate utilization.

Supposedly equivalent energy intakes, whether measured or calculated, cannot be assumed to be physiologically equivalent in providing energy to the animal (Donato, 1987). The diet source must be considered. In many studies, a vegetable oil provided 100% of fat calories: soybean oil

(Toyomizu et al., 1982, 1985), corn oil (Hartsook et al., 1973), or a partially saturated vegetable oil (Salmon and Flatt, 1985). Although absorption might be improved compared to more saturated sources, providing vegetable oil as the sole source of fat is not representative of human diets. In the present study, the fat source was hydrogenated beef tallow and soyabean oil at a P:S ratio of approximately 0.2, and the carbohydrate source was cornstarch and dextrose at a ratio of approximately 1:1. These sources and ratios were chosen to be representative of a "typical" North American diet (Sabry et al., 1984; Van den Reek et al., 1986). Although the highly saturated high fat diet was less well absorbed, the total daily metabolizable energy for both diets was still well above the maintenance requirements of 106 kcal ME/wt $(kg)^{0.75}$ (NRC, 1978). This value equals 42.9 kcal/d for a 300 g rat and 58.2 kcal/d for a 450 g rat. Weight gain was similar between groups per kcal ME. This explains the difference in weight gain previously reported over a range of six carbohydrate to fat ratios from 0.5 to 3.0 (Chapter 5). However, energy gain (per kcal ME) was higher in the group fed the high carbohydrate diet (Cho:Fat 2.0), suggesting that the composition of the gain and other metabolic processes were different due to the energy substrate provided. This was also observed previously (Figure 5.3), where the high fat diet group had carcass energy of 56% fat and 44% protein calories, compared to carcasses of the high carbohydrate diet group, which were 78% fat and 22% protein. Jeejeebhoy (1981) calculated the composition of synthesized tissue from data of nitrogen balance and energy intake of healthy male subjects fed high carbohydrate and high fat diets (Richardson et al., 1979). He estimated that more nitrogen was laid down per kcal with the higher fat diet, as tissue calculated to contain 36% lean and 64% fat. By contrast, with the high carbohydrate diet, tissue composed of 22.3% lean and 77.7% fat was deposited (Jeejeebhoy, 1981). When amino acids and hypertonic dextrose were infused, patients gained body

fat and body water (Yeung et al., 1979), whereas patients receiving amino acids and a mixture of carbohydrate and lipid gained body nitrogen proportionately with body weight gain (Mernagh et al., 1981). Thus, there appears to be a diet composition effect on the partitioning of stored energy.

There is a large difference in the substrate mix provided by the two diets fed. When fat is supplied at 52% of total energy, it is expected that adaptations occur to metabolize the excess fatty acids. Gomez et al. (1972) reported that infusion of lipid was followed by increase in lipid oxidation and decrease in carbohydrate oxidation shown by indirect calorimetry in human subjects. This was mediated by an elevation in serum total glycerol and plasma free fatty acids. Increased lipid oxidation was also observed when subjects were fed a high fat diet of regular foods (Chapter 4). Rats adapted to a high fat diet for 4 to 6 weeks showed enhanced oxidation of [I-C^{14}]-palmitic acid in vitro in liver and muscle, compared to rats adapted to a high carbohydrate diet (Tepperman et al., 1956). Increased fat oxidation was observed in response to increased plasma free fatty acid concentrations in vitro in rat muscle (Eaton and Steinberg, 1961), and in vivo in dogs (Pavle et al., 1966). It is known that fat oxidation inhibits glucose oxidation (Randle et al., 1966; Gomez et al., 1972). Perhaps it also inhibits amino acid oxidation, thereby sparing nitrogen.

It has been reported that septic or injured patients are more likely to oxidize fat, possibly due to an obligatory requirement (Wolfe et al., 1979; Askanazi et al., 1980). Thus, fat may have a special role in the deposition of lean tissue because of an obligatory need for fat oxidation (Wolfe et al., 1979). Flatt (1987) has stated that fat oxidation is not determined by fat intake, but many experiments cited were short-term. Perhaps an adaptation period is necessary for fat oxidation to be stimulated and for mitochondrial enzyme and extramitochondrial changes to occur.

A portion of the energy produced by the animals may be "wasted" due to adaptive thermogenesis. The critical temperature of the fasting rat, the environmental temperature below which heat production must be increased to maintain body temperature, is 30°C (Brody, 1945; Kleiber, 1975). These rats were housed at 21 - 23°C, thus requiring heat production for thermoregulation. A small amount of brown adipose tissue may have resulted in both groups, but it is unlikely that the composition of the diet would have significantly altered brown adipose tissue activity.

Approximately 50% of heat production above maintenance can be accounted for by the metabolic processes required for body fat and protein deposition (Reeds et al., 1982). In the calculations in the present study (Table 6.7), it would account for almost the entire heat production above basal, because only a very small, if any, thermic effect of feeding or exercise would have been measured. The rate of fat synthesis is similar to the rate of fat deposition, but the relationship between protein synthesis and protein deposition is not fixed (Reeds et al., 1982). The rate of protein synthesis may exceed the rate of accretion, resulting in increased energy costs. It is conceivable that the high fat diet resulted in a higher rate of protein synthesis. Because protein synthesis decreases with fasting (Rennie et al., 1982), the heat production measurements would not be representative of the maximum potential energy output from this source.

On the basis of metabolizable energy, the two diet treatments resulted in the same weight gain but a much different energy gain. Increased fat oxidation may be involved in the partitioning of energy storage. Unaccounted-for energy loss in the high fat diet may be due to an increased rate of protein synthesis. This will be examined in the next experiment (Chapter 7).

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Chapter 7.
Protein Synthesis Rates in Tissues of Rats fed Diets with High and Low Ratios of Carbohydrate to Fat

Introduction

A gain in body weight is composed of variable proportions of protein, fat and water. Many factors influence the partitioning of these components, including stage of development, health status, exercise and diet. Recent studies have shown that diet composition affects the composition of body weight gain (Chapters 5,6; Macfie et al., 1981; Jeejeebhoy, 1981; Shulman et al., 1984). Feeding a high fat diet resulted in a greater proportion of protein gain (per total energy gain), whereas feeding a high carbohydrate diet resulted in a greater proportion of fat and/or water gain.

A change in body protein mass is the net result of both protein synthesis and degradation. For protein to be gained, synthesis must be greater than degradation. However, the relationship between protein synthesis and protein deposition is not fixed (Reeds et al., 1982). The rate of protein synthesis (and degradation) is much greater than the rates of either protein intake or deposition (Reeds and Fuller, 1983) and thus, protein synthesis is very expensive in terms of energy requirements (Hegsted, 1974). It can account for approximately 20% of total heat production (Reeds et al., 1981) and a change in the rate of protein synthesis corresponds with a change in metabolic rate (Garrow, 1985).

Thus, the purpose of the present study was to explain the unaccounted-for energy expenditure observed in rats fed a high fat diet (Chapters 5,6) by determining if feeding the high fat diet increased the rate of protein synthesis in tissues of adult male rats. Nitrogen balance studies were also completed to compare protein synthesis and nitrogen retention.

Materials and Methods

Animals and experimental design

Forty-two adult male Sprague-Dawley rats obtained from Biological Sciences Animal Service, University of Alberta, Edmonton, weighing 309 ± 26 g, were used. The rats were divided into two treatment groups containing 21 animals each. These animals were housed individually in metabolic cages for quantitative collection of feces and urine. Animals were kept on a 12-hour light/dark cycle at 24°C.

The rats were fed diets similar in content of gross energy and all essential nutrients for six weeks. The diets differed only in the ratio of dietary carbohydrate to fat (Cho:Fat). The two groups received diets which provided dietary carbohydrate to fat ratios of 0.5 and 2.0 (Table 6.1). Dietary protein was supplied in both diets at a calculated level of 22% of total metabolizable energy. Because of the different fat content in the diets, the caloric densities were different (Cho:Fat 0.5 = 5.43 kcal gross energy/g diet; Cho:Fat 2.0 = 4.44 kcal/g), determined by bomb calorimetry (Model #1241, Parr Instrument Co., Moline, IL). Thus the diet providing a Cho:Fat ratio of 2.0 was provided to the animals in amounts multiplied by a factor of 1.22 of the intake of the Cho:Fat ratio of 0.5 diet group to ensure equal nutrient intakes. Food intake was measured every two days, and food spillage was monitored. Water was provided ad libitum.

Animals were weighed weekly. During the final eight days, 48-hour urine and fecal samples were collected. Urine, preserved with dilute (0.5 N) H_2SO_4, and feces were frozen at -30°C until further analysis. The study was approved by the Animal Policy and Welfare Committee, University of Alberta.

Analytical procedures

After completion of the 6-week feeding trial, protein synthesis rates in liver, muscle and intestine were measured by the method of Garlick et al. (1980) adapted by McAllister (1987). Each rat was given an intraperitoneal injection of a flooding dose of L-[2,6-^{3}H]-phenylalanine (Amersham International, Amersham, U.K.) (150 µmol/ml or 86 µCi/ml) in the amount of 1 ml/100 g body weight. Injections were alternated between treatment groups and animals were sacrificed by decapitation at 3 time points (5, 13 and 20 minutes; n = 7 per group). This time period was previously determined as appropriate for a plateau to be reached (McAllister, 1987).

The carcasses were put in ice water and the liver, gastrocnemius muscle and approximately 60 cm of small intestine flushed with ice water were immediately removed, weighed, frozen in liquid N_2 and stored at -30°C.

Samples were prepared similarly to the methods reported elsewhere (Garlick et al., 1980; McAllister, 1987) (Appendix IV).

Phenylalanine was isolated by enzymatic decarboxylation to ß-phenethylamine followed by organic extraction. Extracts were analyzed by liquid scintillation counting for radioactivity (Beckman LS5801 liquid scintillation spectrometer, Beckman Inc., Fullerton, CA) and by high-performance liquid chromatography (Varian model 5000 coupled to a Fluorichron detector, Varian Inc., Georgetown, Ont.) for phenethylamine content. From these values, the fractional rate of protein synthesis for each tissue was calculated.

Nitrogen excretion in feces and urine was determined for each animal during the last 8 days of the study. Nitrogen analysis was done by a modified Kjeldahl method (Kjeltec System, Tecator, Hoganas, Sweden). Duplicate aliquots were analyzed.

The results of the two treatments were analyzed by t-test statistics (Steel and Torrie, 1980).

Results

The difference in actual food intake between the two diet groups (Table 7.1) was very similar to previous experiments with the same diet treatments (Chapter 5,6) and there was no difference in protein intakes between groups. The gross energy intake was higher for animals fed the 0.5 ratio diet ($p < 0.01$). There was considerable daily within-group variation in intake, which was difficult to control because of the large number of animals in each group.

Table 7.1
Protein and energy intakes of rats fed diets providing different ratios of carbohydrate to fat

	Cho:Fat Ratio	
	0.5*	2.0*
Food intake (g/d)	20.6 ± 0.2†	24.3 ± 0.2
Protein intake (g/d)	5.14 ± 0.05	5.17 ± 0.04
Gross energy intake (kcal/d)	111.7 ± 1.0‡	107.7 ± 0.7

n = 21 per group

* Caloric density of each diet is 5.43 kcal/g and 4.44 kcal/g for the Cho:Fat ratios of 0.5 and 2.0 respectively.

† Mean ± SEM.

‡ Different from 2.0 ratio ($p < 0.01$).

The results of the balance study show that nitrogen intake and excretion was similar between groups (Table 7.2). The group fed the diet providing a Cho:Fat ratio of 2.0 compared to 0.5 had a higher nitrogen balance per gram of body weight ($p < 0.05$). The group fed the Cho:Fat ratio of 0.5 compared to 2.0 had higher nitrogen balance as a function of energy gain, based on calculations from previous experiments (Chapters 5,6). The latter did not reach statistical significance due to considerable within-group variation.

Mean weekly body weights of the treatment groups are shown (Table 7.3). The animals fed the high carbohydrate diet gained more weight than those animals fed the high fat diet. The difference in body weight between groups was significant at weeks 3 ($p < 0.05$), 4, 5, and 6 ($p < 0.01$).

Table 7.2
Parameters of nitrogen metabolism

	Cho:Fat Ratio	
	0.5	2.0
Nitrogen intake:		
(mg/d)	823 ± 13*	826 ± 12
Nitrogen excretion:		
(mg/d)		
urinary N	675 ± 15	623 ± 26
fecal N	58 ± 4	53 ± 3
Nitrogen balance:		
(mg N /g body wt)	.205 ± 0.035†	.330 ± 0.038
(mg N /kcal daily energy gain)	13.640 ± 2.61	9.480 ± 1.20
(mg N /total energy gain)	.320 ± 0.06	.230 ± 0.03

n = 21 per group
* Mean ± SEM.
† Different from 2.0 ratio ($p < 0.05$).

Table 7.3
Body weights of rats fed diets providing different ratios of carbohydrate to fat

Cho:Fat Ratio	Week						
	0	1	2	3	4	5	6
0.5	309* ± 6	325 ± 7	343 ±7	372† ± 9	384‡ ± 8	400‡ ± 9	422‡ ± 7
2.0	309 ± 6	328 ± 4	355 ± 4	395 ± 6	423 ± 7	440 ± 7	462 ± 7

n = 21 per group
* Mean ± SEM.
† Different from 2.0 ratio ($p < 0.05$).
‡ Different from 2.0 ratio ($p < 0.001$).

The difference in tissue weights (Table 7.4) between groups corresponded to the difference in body weight. That is, the rats fed the high carbohydrate diet had higher tissue weights for liver ($p < 0.05$) and muscle ($p < 0.001$). There was no difference in intestine weights between groups.

Table 7.4
Tissue weights of rats fed diets providing different ratios of carbohydrate to fat

Tissue Weights (g)	Cho:Fat Ratio	
	0.5	2.0
Liver	12.6 ± 0.3*†	14.9 ± 0.4
Small intestine	6.4 ± 0.1	6.2 ± 0.2
Muscle (gastrocnemius)	2.8 ± 0.1‡	3.0 ± 0.1

n = 21 per group
* Mean ± SEM.
† Different from 2.0 ratio ($p < 0.05$).
‡ Different from 2.0 ratio ($p < 0.001$).

Table 7.5 shows the specific radioactivity of free phenylalanine in tissues at the three time points after injection of ^{3}H-phenylalanine. The large dose given resulted in rapid flooding of all precursor pools and a relatively constant plateau for all tissues over the time points chosen (Appendix V). Thus, the mean intracellular specific radioactivity (time 5 to 20 minutes) was used for the calculation of fractional rate of protein synthesis. Only in the liver

Table 7.5
Specific radioactivity of intracellular phenylalanine in tissues of rats at different times after injection of L-[2,6 ^{3}H]-phenylalanine

Cho:Fat Ratio	Time after injection (min) 5	13	20	Mean (5 - 20 min)
	Specific Radioactivity (dpm/nmol)			
Intestine:				
0.5	844 ± 12 [a]	1090 ± 51 [a]	912 ± 167 [a]	965 ± 64 (n = 17)
2.0	947 ± 87 [a]	1005 ± 53 [a]	857 ± 86 [a]	927 ± 46 (n = 17)
Muscle:				
0.5	741 ± 35 [a]	924 ± 35 [b]	941 ± 44 [b]	876 ± 30 (n = 17)
2.0	685 ± 34 [a]	950 ± 17 [b]	953 ± 30 [b]	868 ± 33 (n = 19)
Liver:				
0.5	696 ± 86 [a]	670 ± 24 [a]	578 ± 39 [a†]	645 ± 31 † (n = 17)
2.0	744 ± 35 [a]	772 ± 49 [a]	694 ± 45 [a]	734 ± 23 (n = 18)

* Mean ± SEM.
a Values not sharing a common superscript letter are significantly different ($p < 0.05$).
† Different from 2.0 ratio ($p < 0.05$).

was there a diet effect on intracellular specific radioactivity, which was lower in the animals fed the high fat diet ($p < 0.05$) at 20 minutes after injection, and for the overall mean of all time points. The intracellular specific radioactivity (S_a) was calculated as:

$$S_a = \frac{^3\text{H-PEA} \times 10}{\text{PEA}}$$

$$\text{Intracellular Specific Radioactivity} = \frac{\text{radioactivity labelled phenethylamine} \times 10 \text{ (dpm/ml)}}{\text{total phenethylamine (nmol/ml)}}$$

The phenethylamine represents the amount of phenylalanine present before the enzymatic conversion with tyrosine decarboxylase. Random samples of each tissue were analyzed for background phenethylamine by eliminating the enzyme step of the assay. No background phenethylamine was found in liver or muscle samples. Intestinal samples had small amounts present (3.96 ± .49 and 2.30 ± .19 nmol/ml in the 0.5 ratio and 2.0 ratio diet groups respectively). This mean value was subtracted from each of the samples from the respective groups before specific radioactivity was calculated.

Protein-bound specific radioactivity (S_b) was calculated by the same formula as the intracellular fraction where:

$$S_b = \frac{^3\text{H - PEA}}{\text{PEA}}$$

The labelled portion is not multiplied by 10 because 1 ml of protein hydrolysate extract was added to the scintillation vials for counting, whereas only 100 µl of the intracellular extract was used. The protein-bound specific radioactivity showed a linear response over time in all tissues (Figure 7.1) However, there was no effect of diet observed. Fractional rates of protein synthesis (Table 7.6)

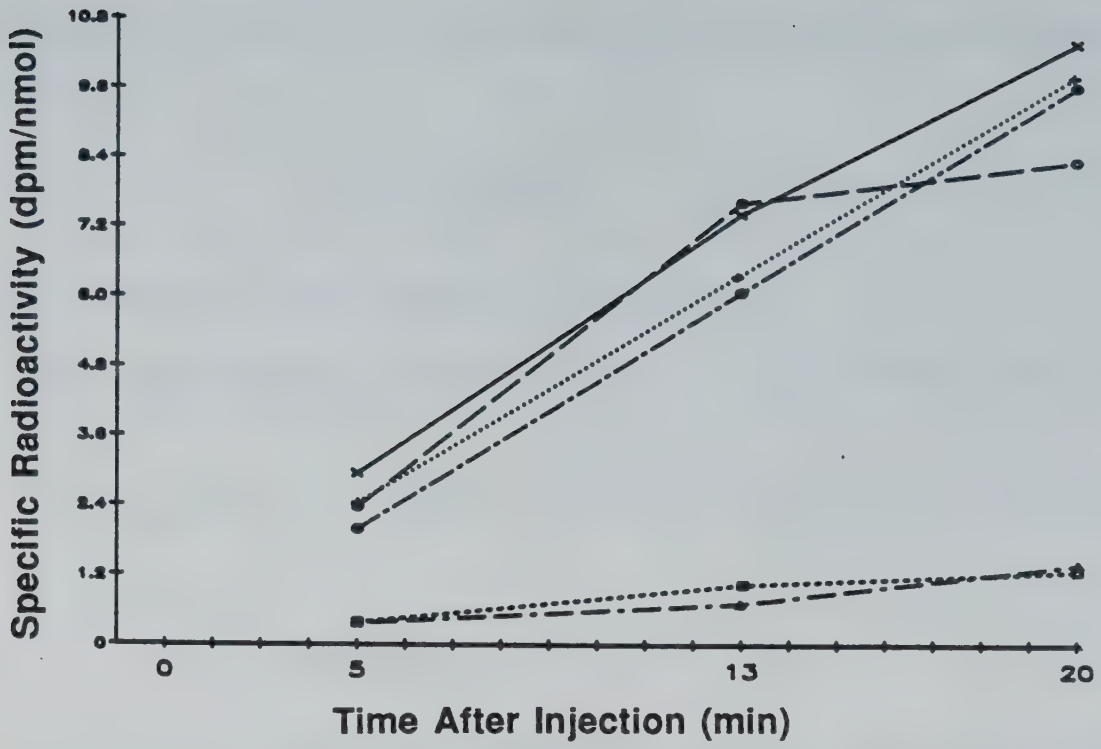

Figure 7.1 Protein-bound specific radioactivity in tissues of rats fed diets providing different ratios of carbohydrate to fat.

- ×——× small intestine; animals fed Cho:Fat 0.5 (range of SEM = 4-7%).
- o— —o small intestine; animals fed Cho:Fat 2.0 (range of SEM = 7-17%).
- +·····+ liver; animals fed Cho:Fat 0.5 (range of SEM = 6-10%).
- ●—·—● liver; animals fed Cho:Fat 2.0 (range of SEM = 4-9%).
- □·····□ muscle; animals fed Cho:Fat 0.5 (range of SEM = 11-27%).
- ▲—·—▲ muscle; animals fed Cho:Fat 2.0 (range of SEM = 10-26%).

Table 7.6
Fractional rates of protein synthesis in tissues of rats fed diets providing different ratios of carbohydrate to fat

Tissue	Fractional Synthesis Rate (%/d)* Cho:Fat Ratio 0.5	Cho:Fat Ratio 2.0
Liver	74.4 ± 7.3†	62.3 ± 7.8
Small intestine	109.8 ± 14.6	100.8 ± 5.9
Muscle (gastrocnemius)	9.8 ± 3.7	11.4 ± 1.7

n = 21 per group

* K_s = (%/d) = $\frac{S_b \times 100}{S_a \times t}$ (K_s = fractional rate of protein synthesis; S_b = protein-bound specific radioactivity; S_a = intracellular specific radioactivity; t = time)

† Mean ± SEM.

were calculated for the 15 minutes between the 5 and 20 minute injections as follows:

$$K_s = \frac{S_b \times 100}{S_a \times t}$$

where K_s = fractional rate of protein synthesis (%/d)

S_b = protein-bound specific radioactivity for 15 minutes (5 min. to 20 min.) (dpm/nmol)

S_a = intracellular specific radioactivity (mean of 3 time points) (dpm/nmol)

t = time (d)
i.e., $\frac{\text{15 min. plateau}}{\text{1440 min./day}}$ = 0.01042d.

Rates of protein synthesis in the intestine and the liver of the animals fed the high fat diet (Cho:Fat 0.5) were 16% and 8% higher (N.S.) than for animals fed the high carbohydrate diet (Cho:Fat 2.0), but the opposite trend was observed in the muscle (14% lower). Alterations in diet composition did not appear to result in differences in protein synthesis rates.

Discussion

Improvement in indices of nitrogen retention has been reported when feeding a high fat diet (Chapters 3,5; Forbes et al., 1946; Macfie et al., 1981). A mechanism for this relationship has not been elucidated. The purpose of this study was to examine the effect of diet composition on the rate of protein synthesis in three tissues representative of a large percentage of daily whole body protein synthesis (liver - 24%, skeletal muscle - 25%, and small intestine - 15%) (Waterlow, 1984). This was done to determine if protein synthesis is stimulated by feeding a high fat diet. Also, protein synthesis is an energy-requiring process (Reeds and Garlick, 1984; Waterlow, 1984), and it may be

the source of unaccounted-for energy loss previously observed when rats were fed a high fat diet (Chapter 6).

The animals in the present experiment responded similarly to those in previous experiments (Chapters 5,6) in terms of diet intake and body weight gain when the same diet treatments were provided. There was a smaller difference in nitrogen balance calculated as a function of energy gain in this report, but the trend was the same. Significantly larger liver and muscle weights were observed in animals fed the high carbohydrate diet. This may be due to excess fat deposition in these tissues in accordance with previously reported excess body fat composition in animals fed the same diet treatment (Chapter 5). Although a subjective observation, tissues (particularly the liver tissues) from the carbohydrate-fed group appeared to have a very high fat composition during homogenization.

Rates of protein synthesis showed no differences between treatments in the liver, muscle or intestine. Few investigators have examined the effect of the composition of non-protein energy on protein synthesis or protein turnover. The influence of protein intake and/or energy intake has been studied more extensively. In general, there appears to be a threshold level of protein intake, at approximately maintenance requirement, below which synthesis rate falls, and above which little change occurs (Waterlow, 1984; Motil et al., 1981; Golden et al., 1977). Studies where protein is held constant and energy is altered have shown only small changes in whole body protein synthesis with increased energy intakes (Jackson et al., 1983; Young et al., 1983).

In humans, Motil et al. (1981) examined diet composition effects on protein turnover, but only at excess energy intakes. Subjects were fed maintenance diets, which were then supplemented with carbohydrate and fat in isocaloric amounts, carbohydrate only or fat only to provide intakes of 25%

above maintenance. Pooled data revealed a higher rate of protein retention due to the excess energy, especially when the carbohydrate supplement was fed. This was a result of reduced amino acid oxidation and slightly enhanced protein synthesis. When male subjects were fed diets at maintenance energy intakes (Young and Bier, 1981) protein synthesis increased slightly and amino acid oxidation decreased slightly in the group fed a mixed diet of carbohydrate and fat compared to those fed a high carbohydrate diet. This response of protein turnover may be the explanation for the higher nitrogen balance values observed in healthy male subjects (Chapter 3) fed a high fat versus a high carbohydrate diet. Measurements of whole body protein synthesis represent the net synthesis of all tissues and it is not known which tissues are most affected by diet.

Diets differ in the efficiency with which they can make high-energy phosphate bonds to support anabolism. Thus, it is implied that there would be differences between diets in energy required to deposit protein and fat (Pullar and Webster, 1977). These differences have been delineated for fat deposition (ARC/MRC Committee, 1974; Table 6.7; Reeds et al., 1982), but not yet for protein deposition. It is necessary to determine the extent to which processes contributing to heat production, which may be linked directly or indirectly to protein synthesis, vary with the nature of the diet (Reeds et al., 1981).

In growing pigs, daily nitrogen retention increased 1.21 g N with fat, and 1.14 g N with carbohydrate supplementation per MJ increase in daily metabolizable energy intake. Values for body protein synthesis (g N/kg $W^{0.75}$/d) were 6.14 and 6.42 for the fat- and carbohydrate-supplemented groups. The relationship between protein synthesis and protein deposition is not fixed. Only in a protein-supplemented group was a large proportion of the energy cost of additional protein deposition attributed to protein synthesis

(Reeds et al., 1981). It has been suggested that protein has a greater influence on protein synthesis than does dietary non-protein energy (Reeds and Garlick, 1984).

In lean Zucker rats, at two ages, the older rats (25 days) had a 23% higher daily protein synthesis rate (g protein/kg$^{.75}$) and a 13% higher daily heat production (kJ/kg$^{.75}$/d) than younger rats (18 days) (Reeds et al., 1982). Also, in lean Zucker rats at two body weights, the heavier rats (350 g) had a 53% higher body protein content and 36% higher maintenance heat production than lighter rats (200 g) (Pullar and Webster, 1977). Heat production appears to be more closely related to body protein content than protein synthesis rates. A very large change in synthesis rates in individual tissues would be necessary to influence whole body protein synthesis and therefore energy expenditure.

Situations where lipid fuels (free fatty acids and ketones) are elevated in the plasma include starvation, sustained aerobic exercise (Newsholme and Leech), and feeding a high fat diet (Askew et al., 1975). Lowell and Goodman (1987) investigated protein-sparing in rat skeletal muscle during starvation. Lipid metabolism in vivo was inhibited by administration of nicotinic acid and tetradecylglycidate, resulting in accelerated muscle protein loss due to decreased protein synthesis and increased breakdown. The authors concluded that lipid fuels may modulate protein metabolism (maintaining synthesis and preventing rapid breakdown) in muscle during starvation. This may be unrelated to protein oxidation.

With sustained exercise, an increase in synthesis and a smaller increase in breakdown resulted in net muscle protein deposition (Goldspink et al., 1983), but this varies with muscle function and fiber type (Millward, 1980).

It is not known if this is directly influenced by nutrient supply, but the muscle oxidizes more fat during prolonged exercise (Felig and Wahren, 1975).

A high fat diet fed to rats compared to a high carbohydrate diet resulted in increased plasma ketone concentration and increased concentration of enzymes required for ketone utilization and synthesis (Askew et al., 1975). Enzymes affected were 3 oxoacid CoA transferase in muscle, acetoacetyl CoA thiolase in heart and liver, and hydroxymethylglutaryl CoA synthetase in liver. High concentrations of ketones can inhibit the rate of protein degradation in muscle (Newsholme and Leech, 1986; Wu and Thompson, 1987). Thus, in the three situations cited where lipid fuels are elevated, protein is spared due to the effect on one or more aspects of protein turnover.

Changes in protein mass can occur in a number of ways, the simplest being an increase in the rate of protein synthesis or a decrease in protein breakdown. Combinations of these two extremes are equally likely (Reeds and Garlick, 1984). They are discrete processes which may be regulated in different ways and it is important to determine to what extent the different macronutrients play a role in their regulation.

It appears that diet composition effects on protein metabolism cannot be explained solely by measurements of protein synthesis rates, and that large differences would be necessary to alter whole body energetics. Diet, like other metabolic stimuli, inevitably affects protein synthesis, degradation and oxidation simultaneously. Inhibition of protein oxidation (due to increased fat oxidation, Chapter 4) and decreased protein degradation in some tissues (due to increased ketone utilization) may result as a consequence of high fat feeding. This situation would increase protein retention, but would not be detectable by measuring only the rates of protein synthesis.

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Chapter 8.
Summary and Conclusions

Review of Hypotheses

The non-protein energy source in the diet influences protein and energy metabolism in a number of ways. The hypotheses tested by this research were:

1. Whole body nitrogen retention is influenced by diet composition. When the non-protein energy component of the diet is supplied as a low carbohydrate to fat ratio (high fat diet) versus a high carbohydrate to fat ratio (high carbohydrate diet), protein-sparing is enhanced.
2. Alterations in nitrogen retention occur as a result of changes in available energy due to diet composition. When the non-protein energy component of the diet is supplied as a low carbohydrate to fat ratio versus high —
   (a) energy expenditure decreases
   (b) fat oxidation increases
   (c) fat deposition increases
   (d) heat loss decreases.
3. Alterations in nitrogen retention occur as a result of hormonal changes due to diet composition. When the non-protein energy component of the diet is supplied as a low carbohydrate to fat ratio versus high, the balance of anabolic and catabolic hormones favors protein synthesis.
4. Increased net nitrogen retention occurs as a result of increased protein synthesis.

Hypothesis #1 was verified (Chapter 3) by demonstrating that adult male subjects had significantly higher daily values for nitrogen balance (mg N/kg body weight) when fed diets with a carbohydrate to fat ratio of 1:1 compared to 2:1 at maintenance energy intakes. The hypothesis was also supported in animals (Chapter 5) by demonstrating that adult male rats had significantly higher daily values for nitrogen balance (mg N/kcal energy gain), when fed diets with a carbohydrate to fat ratio of 0.5 compared to 2.0 and 2.5. There was a consistent decrease over this range of ratios. Carcass protein gain (% of energy gain) was significantly higher in rats fed the carbohydrate to fat ratios of 0.5 and 1.5 compared to 2.0. However, animals fed the diets highest in carbohydrate (Cho:Fat 2.5, 3.0) showed increasing values for nitrogen balance and protein gain. At these ratios, there appeared to be a plateau effect or adaptation to prevent further excretion of nitrogen.

In the human study, the same effect was not observed at 75% maintenance energy intakes. Negative energy balance may have over-ridden the effect of diet composition, and stimulated mechanisms for nitrogen conservation.

The remaining hypotheses investigated possible mechanisms for the effect of diet fat on protein-sparing. Consistent trends to support hypothesis #2 (a) were observed in humans, where adult male subjects exhibited a lower resting energy expenditure at both maintenance and 75% maintenance energy intakes when fed diets with a carbohydrate to fat ratio of 1:1 compared to 2:1. The hypothesis was supported in animals (Chapter 6), where adult male rats had significantly lower values for 24-hour energy expenditure as absolute values (kcal/rat/hour) when fed a diet with a carbohydrate to fat ratio of 0.5 compared to 2.0. Although the amimals fed the 0.5 ratio diet had lower weight

gains, the difference in energy expenditure remained significant when corrected for body weight (kcal/g/hour).

Hypotheses #2 (b) was verified in humans (Chapter 4) by demonstrating that adult male subjects had consistently higher values for fat oxidation (i.e., lower respiratory quotients) at rest and at four postprandial time points, when fed diets with a carbohydrate to fat ratio of 1:1 versus 2:1 at maintenance energy intakes. At 75% maintenance energy intakes, the same trend was observed with significantly greater fat oxidation at 0.5, 1.5 and 2 hours postprandial, when the high fat diet was fed.

Hypothesis #2 (c) was not verified in animals (Chapter 5). In this regard, adult male rats demonstrated significantly lower values for fat deposition (% of energy gain) when fed diets with carbohydrate to fat ratios of 0.5 and 1.5 compared to 2.0. Rats also showed significantly lower values for carcass energy deposition (Chapter 6), when fed diets with a carbohydrate to fat ratio of 0.5 compared to 2.0. This remained significantly different when corrected for metabolizable energy, and it is inferred that the higher energy gain for animals fed the diet ratio of 2.0 was primarily due to a higher fat gain. Inconsistent reports regarding fat intake and fat deposition may be due to differences in the type of fat provided (different digestibilities), the amount of fat provided (ad libitum versus controlled feeding regimens), and the stage of development of the rat (weanling versus adult).

Because of experimental constraints, hypothesis #2 (d) was only tested in two animals per diet group (Chapter 6), and no consistent results were observed.

Hypothesis #3 was tested in humans (Chapter 3), where adult male subjects were fed diets with carbohydrate to fat ratios of 1:1 versus 2:1. The nitrogen-sparing effect of the high fat diet did not appear to be explained by

hormone levels observed in the sampling protocol utilized. Many trends were shown, and it may be speculated that hormones play an indirect role in nitrogen retention, but the direct effect appears to be substrate-mediated.

Hypothesis #4 was tested in animals (Chapter 7), where protein synthesis rates in three tissues were measured. No differences in rates of protein synthesis were observed. It is conceivable that diet composition elicits small changes in all components of protein turnover (synthesis, degradation, and oxidation), and that the combined response would alter net nitrogen retention.

This type of research is difficult to implement because of the numerous variables which affect energy and/or protein metabolism. In humans, factors that must be considered include physical activity, body size and composition, age, climate and other ecological factors (FAO, 1973), and health status. In the human metabolic study (Chapters 3,4), these variables were controlled. Thus, the results observed were apparently true effects of diet composition. Furthermore, there are no other research reports regarding this question where regular foods were consumed. In the past, formula diets, supplements, and enteral or parenteral products have been evaluated. The present study provides a situation which represents "normal" physiological response to changes in diet composition. Further information in this regard is necessary before "abnormal" situations (i.e., disease states) can be systematically investigated.

In animals, variables to consider include age, sex, strain, feeding mode, exercise and environment. These factors are generally controllable within an experiment, but wide variations occur in comparisons between experiments.

The largest difference in the design of animal studies reported is the type of diet fed and the source of macronutrients. It has been shown that diets equal in gross energy yield differences in digestible and metabolizable energy (Chapter 6). This is a subject of recent concern. "The composition of the diet with respect to the proportion of the major nutrients...has a profound and variable effect on the efficiency of food energy use and ultimately on the results of an experiment" (Leveille and Cloutier, 1987). "A calorie from fat is not necessarily physiologically equivalent to a calorie from carbohydrate in terms of efficiency of energy utilization during growth" (Donato, 1987). Thus, metabolizable energy should be measured in all experiments involved with energy and protein metabolism. Also, the type and amount of fat and carbohydrate sources should be similar to proportions found in human diets so that practical application can be inferred. In many of the earlier reports, fat was provided solely as vegetable oil (i.e., corn oil, soyabean oil). This diet composition does not resemble human fat consumption patterns (van den Reek et al., 1986; Sabry et al., 1984).

There is evidence to suggest that high carbohydrate feedings are not without problems as a means of nutritional support in many disease states. High carbohydrate intakes, whether oral or infused, may result in increased respiratory distress (Askanazi et al., 1981) increased fat and/or water gain (Macfie et al., 1981; Yeung et al., 1979) and hyperglycemia (Burke et al., 1979). It has been shown that a large proportion of fat in the diet can reduce the respiratory load (i.e., decrease CO_2 production) (Chapter 4), increase the proportion of protein gain (Chapter 5), and because of decreased carbohydrate intake, reduce risk of hyperglycemia. Therefore, a diet high in fat may correct clinical problems associated with high carbohydrate intakes. It is

also suggested that high-fat feedings could lead to improvement in patients with high nitrogen losses.

Future Research

1. Energy metabolism appears to be closely linked to protein metabolism. Differences have been shown in whole body substrate oxidation when high and low dietary carbohydrate to fat ratios are fed (Chapter 4). The next step would be to look more specifically at individual tissues and measure the oxidative activity of the mitochondria.

   Purpose: To determine if diets with different carbohydrate to fat ratios affect ATP production and the oxidative requirements of the mitochondria in various tissues, and to assess if certain tissues are affected by diet composition to a greater extent than others. Preliminary work in this area is currently in progress in our laboratory.

2. Differences in nitrogen balance were observed during the last six days of a two-week metabolic study in humans (Chapter 3) and during the last eight days of a six-week feeding trial in animals (Chapter 5). Measurements at different and, ideally, longer time-points would be useful.

   Purpose: To compare changes in nitrogen balance with pre-treatment values. To determine whether changes observed are acute or sustained, and therefore determine the long-term effects of high fat versus high carbohydrate diet treatments.

3. Comprehensive information is required on the digestibility of various sources of dietary fats and combinations of these sources.

   Purpose: To develop diet treatments of equal digestible energy, yet different carbohydrate to fat ratios.

4. Measurements are required of the nitrogen content of various tissues of animals fed diets with high and low carbohydrate to fat ratios. Comparisons then could be made between visceral and carcass nitrogen deposition.

   Purpose: To determine the fate of retained nitrogen.

5. Measurements are required of protein oxidation in tissues (animals) and the whole body (humans) when diets of high and low carbohydrate to fat ratios are fed.

   Purpose: To determine if protein oxidation is inhibited, and to what extent, by lipid fuels in the plasma and subsequent increases in fat oxidation observed with high fat feeding.

Does a high fat diet promote nitrogen retention to a greater extent than a high carbohydrate diet? This question has been asked for more than a century (Voit and Atwater, 1881; Maignon, 1933; Calloway and Spector, 1955; Pineault et al., 1988). We are getting closer to a definitive answer.

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Appendices.

Appendix I.
Experimental Diets (Human Metabolic Study)*

Food servings of experimental diets (Menu — Day 1)*

Food	Maintenance Energy		75% of Maintenance Energy	
	High Carbohydrate	High Fat	High Carbohydrate	High Fat
	Servings (g)			
Breakfast:				
cornflakes	18	18	18	18
whole milk	80	129	258	125
bread (100% ww)	90	60	60	60
margarine	18	28	15	15
jam	30	20	23	21
cream (10% fat)	62	125	-	125
Lunch:				
egg	50	50	50	50
bread (100% ww)	60	60	60	60
mayonnaise	20	25	20	20
margarine	5	22	-	15
peaches (juice packed)	258	258	258	258
plain cookies	35	25	15	10
lemonade	300	105	167	-
Supper:				
spaghetti	222	148	222	222
tomato sauce	125	125	125	147
ground beef	22	30	22	18
margarine	17	28	9	21
salad dressing	20	30	20	20
apple juice	235	278	227	-
raisins	42	14	-	-
green salad	-	-	-	-
Snack:				
cheddar cheese	30	30	30	40
graham crackers	64	56	56	56
margarine	13	32	6	15
banana	228	228	228	-
orange juice	285	100	85	200

* Quantity of food supplied for Menu Day One on four different diet treatments. Sample diet is shown for one subject whose daily requirements were 59 g protein, 3250 kcal energy (at maintenance) and 2450 kcal (at sub-maintenance). Data in Chapters 3 and 4.

Appendix I (continued)

Experimental diets (Menu — Days 2 and 3)

	Menu Day 2	Menu Day 3
Breakfast:		
	rice krispies	sugar crisp
	whole milk	whole milk
	bread (100% ww)	bread (100% ww)
	margarine	margarine
	jam	jam
	cream	cream
	apple juice	apple juice
	banana	
Lunch:		
	cheddar cheese	weiner
	dinner roll	hot dog bun
	mayonnaise	mayonnaise
	margarine	margarine
	sliced tomatoes	orange juice
	fruit cocktail	apple
	lemonade	
Supper:		
	chicken	cheese
	rice	macaroni
	celery	tomato
	carrots	zucchini
	margarine	margarine
	frozen strawberries	pears
	whipped cream	
Snack:		
	instant pudding	chocolate milk
	whole milk	orange juice
	cream	popcorn
	margarine	margarine
	doughnuts	

Appendix I (continued)

Nutrient composition of experimental diets*

Cho:Fat Ratio	Protein	Carbohydrate	Fat	Fiber (crude)	P:S Ratio
	(g)	*(g)*	*(g)*	*(g)*	
Maintenance Energy					
High Carbohydrate	59	505	109	7.1	1.1
High Fat	59	374	168	6.3	1.0
75% of Maintenance Energy					
High Carbohydrate	59	369	81	6.2	0.9
High Fat	59	275	123	4.9	0.8

Energy distribution of experimental diets*

Cho:Fat Ratio	Protein	Carbohydrate	Fat	Total	Cho:Fat Ratio
	(kcal)	*(kcal)*	*(kcal)*	*(kcal)*	
Maintenance Energy					
High Carbohydrate	236	2020	981	3237	2.1
High Fat	236	1446	1512	3244	1.0
75% of Maintenance Energy					
High Carbohydrate	236	1476	729	2441	2.0
High Fat	236	1100	1107	2443	1.0

* Sample diet is shown for one subject whose daily requirements were 59 g protein, 3250 kcal energy (at maintenance) and 2450 kcal (at sub-maintenance). Data in Chapters 3, 4.

Appendix II.
Changes in Postprandial Carbon Dioxide Production after Diet Treatment

Cho:Fat Ratio	CO_2 production (VCO_2 – ml/min)					
	rest*	1/2 h†	1 h	1 1/2 h	2 h	2 1/2 h
Maintenance Energy						
High carbohydrate (2:1)	188 ± 14	234 ± 29	234 ± 34	227 ± 34	228 ± 36	246 ± 25
High fat (1:1)	165 ± 29	212 ± 38	226 ± 39	219 ± 39	210 ± 39	221 ± 40
75% of Maintenance Energy						
High carbohydrate (2:1)	172 ± 16	212 ± 13	216 ± 15	218 ± 21	206 ‡ ± 17	204 ± 17
High fat (1:1)	155 ± 21	200 ± 24	209 ± 18	195 ± 24	188 ± 18	174 ± 20

n=5 ( ) Brackets denote a dietary carbohydrate to fat ratio of 1:1 or 2:1.
Mean ± SEM
* Fasting (12h).
† 1/2 hour after eating.
‡ Different from high fat diet (@ 75% maintenance energy) ($p < 0.05$) (Chapter 4).

Appendix III.
Calculation of Heat Production (Chapter 6)

$$HP\ (kcal/h) = VO_2\ (l/h) \times [(87 + 27\ RQ/22.4]$$

Where:

HP = heat production (kilocalories per hour)
VO_2 = volume oxygen consumed (litres per hour)
RQ = respiratory quotient

$$[(87 + 27\ RQ/22.4]^* = \frac{\text{heat production per mol oxygen consumed } (\Delta Q)}{\text{litres oxygen per mol}}$$

$$^*\frac{\Delta Q}{\text{mol } O_2} = 87 + 27\ RQ\ ^\dagger \text{ (derivation page 374)}$$

$$^*\frac{LO_2}{\text{mol } O_2} = 22.4$$

† Equation based on calculations where it is assumed that the mixture of carbohydrate and fat catabolized contains 1 gram atom of carbon. A fraction of this gram atom of carbon is contained in fat (C_f); the other fraction in carbohydrate (C_s)

Therefore, $C_f + C_s = 1$

$C_f = 2.41/RQ - 2.41$

$C_s = 3.41 - 2.41/RQ$

(derivation page 365-367)

(Klieber, M. (1975) Fire of Life, R.E. Krieger Publ. Co., Huntington, New York)

Appendix IV.
Assay for the Measurement of Rates of Protein Synthesis

1. *Tissue homogenization*

(a) Frozen samples were covered with 2% perchloric acid until partially thawed.
(b) Samples were ground in a tissue homogenizer (polytron) with 2% perchloric acid (which precipitates protein to separate it from free amino acids).
(c) Centrifugation at 2000 rpm for 15 minutes separated the intracellular amino acid fraction (supernatant) from the protein fraction.
(d) The supernatant was removed.

2. *Protein pellets*

(a) Pellets were washed 3 times with 2% perchloric acid and centrifuged each time at 2000 rpm for 15 minutes. The supernatant was discarded.
(b) Pellets were resuspended in 6 N HCl and hydrolyzed for 24 hours at 110°C.
(c) HCl was removed by drying the hydrolysates in a water bath at 100*C with a light stream of nitrogen gas for 24 hours.
(d) Hydrolysates were resuspended in 0.5 M sodium citrate (pH 6.3) as preparation for enzymatic conversion and extraction.

3. *Intracellular fraction*

(a) An aliquot of the intracellular fraction was combined with saturated tripotassium citrate to neutralize the sample solution.
(b) The solution was centrifuged at 2000 rpm for 10 minutes and a white precipitate of potassium perchlorate formed.
(c) The supernatant was used for enzymatic conversion and extraction. The precipitate was discarded.

4. *Enzymatic conversion and extraction*
(to convert phenylalanine to ß-phenethylamine)

(a) The enzyme was prepared in advance as follows:
- L-tyrosine decarboxylase 2U/ml (Sigma # T4379)
- with pyridoxyl phosphate 1 mg/ml (Sigma #P9255)
- in 0.5 M sodium citrate pH 6.3
- and stored at -70°C

(b) 0.5 ml sample of enzyme was added to 1.0 ml of the intracellular fraction and 0.6 ml of enzyme was added to 0.5 ml of the protein hydrolysate.
(c) Samples were incubated overnight at 50°C.

(d) 0.5 ml 3 M NaOH and 5.0 ml chloroform-n-heptane (1:3 v/v) were added to the incubated solution.
(e) Samples were centrifuged at 1200 rpm for 5 minutes.
(f) The top layer was saved and 2.5 ml of chloroform and 2.0 ml 0.1 M H_2SO_4 were added to it. Samples were centrifuged at 1200 rpm for 5 minutes.
(g) The top layer was saved to be used for scintillation counting and HPLC analysis.

5. *Radioactivity Counting*

(a) Final extracts were combined with scintillation fluid:
- 1.0 ml protein fraction + 12 ml scintillation fluid,
- 0.1 ml intracellular fraction + 12 ml scintillation fluid,

and counted to yield dpm/ml.

6. *HPLC Analysis*

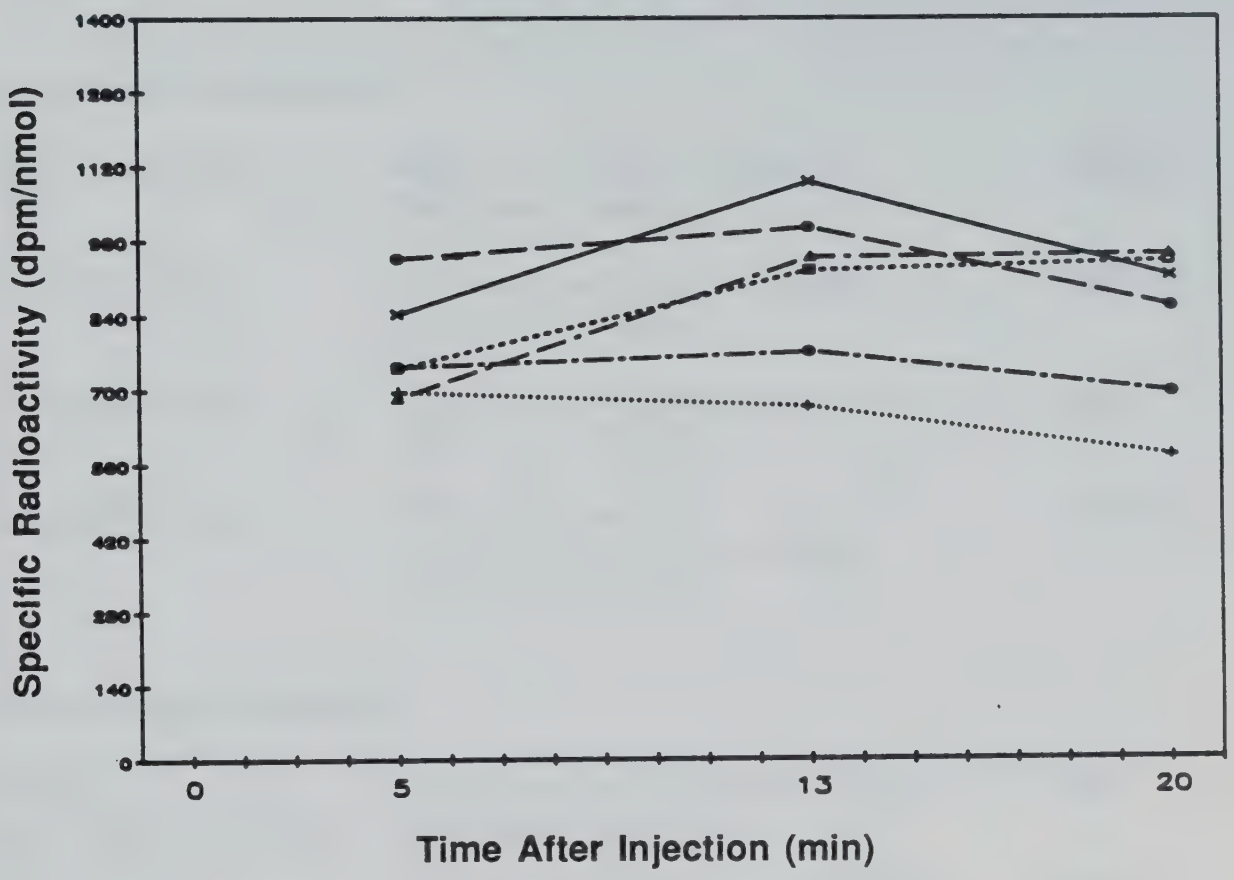
(a) Final extracts were combined with ethanolamine, saturated potassium borate solution and distilled deionized water, and analyzed on HPLC for phenethylamine content (nmol/ml):

10 µl protein fraction	+ 100 µl ethanolamine
	+ 50 µl saturated potassium borate
	+ 1.0 ml water
100 µl intracellular fraction	+ 100 µl ethanolamine
	+ 100 µl saturated potassium borate
	+ 1.0 ml water

Data in Chapter 7.

Appendix V.
Flooding Dose Technique for Measurement of Protein Synthesis: Establishment of Plateaus in Specific Radioactivity of Intracellular Fraction

Rates of protein synthesis in three tissues — liver, small intestine and muscle — were measured in adult rats (Chapter 7). A large single dose of L-[^{3}H]-phenylalanine was injected intraperitoneally to flood the body pools of free amino acids such that the specific radioactivity in the plasma and the tissues were similar. A plateau of the specific radioactivity in the intracellular fraction of all tissues was established between 5 and 20 minutes after injection (figure below).



Intracellular specific radioactivity in tissues of rats fed diets with high carbohydrate to fat ratios at three time-points after injection of ^{3}H-phenylalanine.

- intestine; animals fed Cho:Fat 0.5
- intestine; animals fed Cho:Fat 2.0
- muscle; animals fed Cho:Fat 0.5
- muscle; animals fed Cho:Fat 2.0
- liver; animals fed Cho:Fat 0.5
- liver; animals fed Cho:Fat 2.0

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• Canadian Dietetic Association, Board of Examiners	1988
• Canadian Dietetic Association, Continuing Education Committee	1986-88
• Graduate Curriculum Committee, Department of Foods and Nutrition	1985-86
• Dean's Selection Committee, Faculty of Home Economics	1985-86

Honours and Awards

• Finalist, American Institute of Nutrition Graduate Student Competition	1988
• Elizabeth Russell Maceachran Scholarship	1987
• Alberta Heritage Foundation for Medical Research Studentship	1984-88
• Province of Alberta Graduate Scholarship	1980
• Canadian Dietetic Association Graduate Scholarship	1978
• Beatrice K. Brownlee Award	1977
• Canadian Dietetic Association Undergraduate Scholarship	1977
• Dean's Honour List	1976, 1977
• IBM Scholarship	1976

Publications

McCargar LJ, Baracos VE, And Clandinin MT (1988) Influence of carbohydrate to fat ratio on whole body nitrogen retention and body composition in adult rats. (submitted)

McCargar LJ, Clandinin MT, Belcastro AN and Walker K (1988) Influence of dietary carbohydrate to fat ratio on whole body nitrogen retention, substrate utilization and hormone response in healthy male subjects. (accepted, Am. J. Clin. Nutr.)

McCargar LJ, Clandinin MT, Fawcett DM and Johnston JL (1988) Short term changes in energy intake affect serum insulin, neutral amino acids and urinary catecholamine excretion in women. (in press, Am. J. Clin. Nutr.)

Brown G, Whyte L, Gee M, Crockford P, Grace M, Oberle K, Williams T and Hutchison K (1984) Effects of two "lipid-lowering diets on plasma lipid levels of patients with peripheral vascular disease. J. Am. Diet. Assoc. 84:546-50.

Hutchison K, Oberle K, Crockford P, Grace M, Whyte L, Gee M, Williams T and Brown G (1983) Effects of dietary manipulation on vascular status of patients with peripheral vascular disease. J. Am. Med. Assoc. 249:3326-30.

Abstracts

(All abstracts were presentations at professional meetings.)

McCargar LJ, Baracos VE, And Clandinin MT (1988) Influence of non-protein energy source on nitrogen retention and body composition in mature rats. Faseb J. 2:A642.

McCargar LJ, Clandinin MT and Belcastro AN (1987) Influence of dietary carbohydrate to fat ratio on body composition, substrate utilization and energy expenditure. Can. Diet. Assoc. J. 48:180.

McCargar LJ, Clandinin MT, Belcastro AN and Walker K (1987) Influence of dietary carbohydrate to fat ratio on whole body nitrogen retention and hormone response. Proceedings of the 30th Annual Meeting of the Canadian Federation of Biological Societies 30:98.

Johnston JL, McCargar LJ, Clandinin MT and Fawcett DM (1986) Short term overeating increases serum insulin levels and urinary catecholamine excretion in normal women. Proceedings of the 5th International Congress on Obesity.

McCargar LJ and Johnston JL (1986) Urinary norepinephrine excretion during short term changes in energy intake in normal weight women. Proceedings of the 29th Annual Meeting of the Canadian Federation of Biological Societies 29:124.

Whyte LJ, Oberle KA, Brown GD, Crockford PM, Grace M, Hutchison KJ and Williams HTG (1981) Effects of dietary manipulation on patients with peripheral vascular disease. Can. Diet. Assoc. J. 42:280.

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